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EFFECTS OF TWO PREPARATIONS OF NAPHTHYLACETIC ACID ON THE GERMINATION AND EARLY GROWTH OF WHEAT SEED DAMAGED BY FORMALDEHYDE[1]

By N. H. Grace[2]

Abstract

Two varieties of wheat seed were sprinkled with solutions containing formaldehyde and naphthylacetic acid to give treatments of 1 and 10 parts of the latter to a million parts of seed by weight. In both varieties formaldehyde treatment reduced the germination rate, final germination count, and the air-dry weight of stems and roots at 29 days after planting. Some reduction in root suppression resulted from the 1 p.p.m. concentration of hormone; the higher level had no effect.

The experiment involved the use of two preparations of naphthylacetic acid, differing only in respect to a trace of halogen in one. This impurity had no effect on final germination or stem and root weights, but increased the germination rate. The trace of halogen had the more marked effect at the highest concentration and the variety Marquis was affected to a greater extent than Reward.

A recent communication reported an experiment in which wheat seed was soaked in solutions of formaldehyde and naphthylacetic acid (3). While the results from blotting paper germination indicated some reduction in formaldehyde injury on treatment with naphthylacetic acid, germination and early growth in soil failed to show any reduction in the injury. The present communication describes an experiment in which seed wheat was treated by a sprinkling technique, a method that permits application of a definite weight of plant hormone chemical to a given weight of seed. Further, two preparations of 1-naphthylacetic acid, known to differ slightly, were used. In this manner, additional information was obtained on the response of formaldehyde treated seed to definite amounts of naphthylacetic acid, and the possible significance of traces of other chemicals in the hormone were investigated.

Experimental

Naphthylacetic acid solutions containing 5 and 50 p.p.m. were prepared in a 1 : 320 solution of commercial formaldehyde (37% by weight of the gas). Two different preparations (2, 5) of naphthylacetic acid were used in the investigation, differing only in respect of a trace of halogen shown to be present in one by means of a flame test.

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Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. N.R.C. No. 908.

[2] *Biochemist.*

Two varieties of wheat, Marquis and Reward, were used in an effort to determine whether there was any difference in the response of varieties to either concentration or preparation of naphthylacetic acid. Solutions were applied by the sprinkling method of treatment, 10 cc. to 50 gm. of seed, effecting treatments of 1 and 10 p.p.m. (parts of naphthylacetic acid to one million parts of wheat by weight). The treated seed was held in the manner previously described (3), and planted in sterilized soil in units of 50 seeds in small cardboard flats, approximately 24 hr. after treatment. These were kept in a greenhouse room which ranged in temperature from 60 to 68° F.

The experiment comprised four treatments consisting of an untreated control, a formaldehyde control, and the two treatments with formaldehyde and naphthylacetic acid at levels of 1 and 10 p.p.m. The two varieties were placed at random in duplicate sub-blocks, each a Latin square, within a large block. Treatments were randomized within sub-blocks in the form of double plots (two flats) containing the two different naphthylacetic acid preparations. There were also double plots of the untreated and formaldehyde treated controls in order to give the averages of hormone concentrations and controls the same weight. Each sub-block contained eight replicates of the treatments, or four replicates of the naphthylacetic acid preparations; the entire experiment involved the use of 128 flats. This arrangement provided three levels of precision for the various comparisons; these were, in descending order, between preparations, treatments, and finally varieties.

Seed was planted on March 17, 1939, and daily germination counts were made as soon as plants emerged; counts were continued until germination was virtually complete. The daily counts were used to compute germination rates by the method of Bartlett (1). The plants were washed out of soil 29 days after planting, placed in an oven at 95° C. for two hours, conditioned in the laboratory for a week, and air-dry stem and root weights determined (4). All data on germination rates, final germination counts, and air-dry weights were subjected to analyses of variance.

Results

In Table I are given data from the analyses of variance. There were significant treatment effects with respect to all four characters considered. The germination rates also showed significant differences between the two preparations of naphthylacetic acid, and the interactions between preparations and dosage and preparations and varieties also were significant.

The average germination rates, final germination count, and air-dry weight of stems and roots are given in Table II. In each case formaldehyde alone effected injury. While 1 p.p.m. of naphthylacetic acid did not increase final germination significantly above the formaldehyde control, the value obtained was not significantly below the untreated control. All treatments gave air-dry weights of stems lower than that of the untreated control; and weights with both hormone concentrations failed to differ from the formaldehyde control. While all formaldehyde treatments were below the untreated control

TABLE I

Analysis of variance of response of wheat seed treated with formaldehyde and naphthylacetic acids

Source of variance	D.f.	Mean square			
		Germination rate	Final germination count	Weight of stems	Weight of roots
Blocks between varieties	1	.0795	24.50	.1152	.00173
Within varieties (error (a))	2	.2661	102.58	.0554	.00886
Rows	12	.0291***	89.13*	.0229*	.00344**
Columns	12	.0164**	29.28	.0193*	.00414**
Treatments	3	.0198*	175.55*	.1093***	.05268***
Treatments × squares between varieties	3	.0031	7.69	.0070	.00134
Error (b)	30	.0058	42.10	.0086	.00106
Between preparations	1	.0418**	1.12	.0001	.00080
Preparations × dosage	1	.0215*	36.12	.0030	.00001
Preparations × varieties	2	.0217**	60.62	.0001	.00462
Error (c)	28	.0047	53.55	.0141	.00163

* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*
*** *Exceeds mean square error, 0.1% level of significance.*

TABLE II

Average effects of formaldehyde-naphthylacetic acid treatments on germination and early growth of wheat seed in soil (mean of two varieties)

Treatments	Germination rate	Final germination count, %	Air-dry weight of stems	Air-dry weight of roots
Untreated	.613	41.1	.736	.264
Formaldehyde alone	.572	36.9	.601	.172
With 1 p.p.m. naphthylacetic acid	.608	38.5	.645	.194
With 10 p.p.m. naphthylacetic acid	.564	35.7	.628	.188
Necessary difference, 5% level	.038	3.3	.047	.017

in respect of air-dry weight of roots, both hormone concentrations were above the formaldehyde control, the 1 p.p.m. level to a significant extent.

The average germination rate of both varieties was greater by 0.036 after treatment with the naphthylacetic acid preparation containing a trace of halogen. This difference was more marked at the greater hormone concentration, and was 0.010 at the 1 p.p.m. level and 0.062 at the 10 p.p.m. The varieties reacted somewhat differently to the two preparations, the presence of halogen increasing the average germination rate of Reward by 0.029 and Marquis by 0.043. This varietal effect was in evidence at both concentrations of phytohormone.

Some reduction of formaldehyde injury is indicated at the 1 p.p.m. level of treatment, a result in agreement with earlier findings in which 1 p.p.m.

indolylacetic acid in formaldehyde increased the air-dry weight of stems of plants grown in soil (3). It is interesting to note that traces of halogen impurity failed to affect final germination or air-dry weight of stems and roots but did increase the germination rate. It may be concluded that minor impurities in growth stimulating chemicals can have a significant effect on growth responses.

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VARIETAL DIFFERENCES IN BARLEYS AND MALTS

X. CORRELATIONS OF CARBOHYDRATES WITH NITROGEN FRACTIONS AND WITH MALT EXTRACT, STEEPING TIME, AND MALTING LOSS[1]

By Henry R. Sallans[2] and J. Ansel Anderson[3]

Abstract

Glutelin is the only nitrogen fraction that is significantly correlated with starch, barley extract, and insoluble carbohydrate, between varieties. In each case the correlation coefficient barely attains the 5% level of significance. Within varieties the correlation coefficients for starch and barley extract with glutelin, hordein, and salt-soluble nitrogen are all negative and attain the 1% level of significance. Insoluble carbohydrate shows no intra-varietal associations with any of the nitrogen fractions.

Starch and barley extract are very closely associated with malt extract both within and between varieties. Insoluble carbohydrate is closely related to malt extract between but not within varieties. It is shown that Bishop's principle of regularities in the carbohydrate and nitrogen composition within varieties fails to apply to insoluble carbohydrate. Intra-varietal associations of steeping time with starch, $r = 0.797$, barley extract, $r = 0.730$, and insoluble carbohydrate, $r = -0.782$, are not dependent on the total nitrogen of the barleys.

Regression coefficients of malt extract on barley extract are homogeneous both within and between varieties, and the average varietal and station regressions do not differ significantly. It is shown that barley extract is more closely related to malt extract than either starch or insoluble carbohydrate, between varieties. Within varieties it affords a more accurate estimate of malt extract than either starch or total nitrogen.

Malt extract is composed mainly of the carbohydrate and nitrogenous materials of the barley from which the malt is made. Therefore it appears logical that any successful prediction of malt extract should take cognizance of both these important constituents of the barley. In pursuing the search for relations on which to base such a prediction, the correlations of the carbohydrate fractions of barley with barley nitrogen fractions and with malt extract, steeping time, and malting loss have been investigated. The results of this study are discussed in the present paper.

Part IX (2) of this series of papers dealt with varietal differences in the carbohydrate composition of barleys and their relations with total nitrogen and 1000-kernel weight. In view of the importance of relations between nitrogen and carbohydrate, it appeared advisable to investigate these in greater detail. The present study was, therefore, extended to include correlations with insoluble nitrogen, alcohol-soluble nitrogen, and total salt-soluble nitrogen.

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Data

Experimental data were obtained from 144 samples of barley representing 12 varieties grown at 12 widely separated experimental stations in Canada. Malting methods and summaries of the data on malt extract, malting loss, and steeping time were given in Part IV (6). The data on total, insoluble, alcohol-soluble, and salt-soluble nitrogen were presented in Part I (1) together with a brief description of the barley varieties and the conditions under which they were grown. The methods used and the results obtained for the carbohydrate fractions of barley appeared in Part IX (2).

Correlations between Carbohydrate and Nitrogen Fractions of Barley

The simple inter-varietal and inter-station correlation coefficients between the carbohydrate and nitrogen fractions of barley are shown in the upper half of Table I. The corresponding partial correlation coefficients, independent of total nitrogen, are given in the bottom half of the table. Following the plan of other papers (7, 8) in this series, it seems advisable to discuss the inter-varietal and inter-station relations in separate sections.

TABLE I

SIMPLE AND PARTIAL CORRELATION COEFFICIENTS, INDEPENDENT OF TOTAL NITROGEN, BETWEEN CARBOHYDRATES AND NITROGEN FRACTIONS OF BARLEY

Barley property	Inter-varietal				Inter-station			
	Total nitrogen	Insoluble nitrogen	Alcohol-soluble nitrogen	Salt-soluble nitrogen	Total nitrogen	Insoluble nitrogen	Alcohol-soluble nitrogen	Salt-soluble nitrogen
Simple								
Starch	−.399	−.582*	−.060	.018	−.953**	−.890**	−.939**	−.801**
Barley extract	−.401	−.590*	−.115	.109	−.908**	−.857**	−.897**	−.744**
Nitrogen in extract	.241	−.387	.077	.821**	.924**	.807**	.875**	.966**
Cellulose-lignin	.293	.634*	.012	−.212	−.320	−.188	−.298	−.482
Partial								
Starch	—	−.511	.490	.126	—	−.062	−.004	.065
Barley extract	—	−.519	−.392	.229	—	−.102	−.036	.149
Nitrogen in extract	—	−.330	−.209	.810**	—	.336	−.535	.892**
Cellulose-lignin	—	.591	−.403	−.303	—	.304	.108	−.422

*In this and later tables, ** denotes that the 1% level, and * that the 5% level of significance is attained.*

Inter-varietal Relations

In general, the inter-varietal simple and partial correlation coefficients are very similar in magnitude. The simple coefficients for the correlation of insoluble nitrogen with starch, extract, and cellulose-lignin residue are sig-

nificant, but the partial coefficients just fail to attain the 5% level. However, it is obvious that these simple and partial coefficients do not differ significantly, and hence the effects of total nitrogen are not sufficient to explain the simple correlations between varietal means.

In Part IX (2) it was shown that barleys having a low kernel weight tend to be low in the reserve carbohydrates, starch and barley extract, and high in the structural materials, cellulose and lignin. The correlation coefficients of insoluble nitrogen (structural protein (3)) with starch, extract, and cellulose-lignin residue are similar in magnitude and sign to those obtained with 1000-kernel weight. It appears that there is an association between structural carbohydrates and structural proteins, and as the proportion of these increases there is a compensating decrease in the proportion of reserve carbohydrates. Furthermore, these relations are associated to some extent with the kernel weight of the barley. Apart from these rather loose associations between the carbohydrates and structural protein, there appears to be no other inter-varietal relation between the carbohydrates and the nitrogen fractions of the barleys.

A fairly close relation exists between the nitrogen in the barley extract and total salt-soluble nitrogen. It seems that the barley wort acts in much the same manner as a dilute salt solution, with the result that it contains essentially the same nitrogen fractions as the 5% potassium sulphate extract.

Inter-station Relations

The simple inter-station correlation coefficients in the upper right corner of Table I show that starch and barley extract are negatively associated with all the barley nitrogen fractions and that extract nitrogen is positively associated with them. Since total nitrogen is closely related to these nitrogen fractions (1, 3) and also to starch, extract, and extract nitrogen, partial correlation coefficients, independent of total nitrogen, were computed to eliminate these common associations. These partial coefficients, shown in the lower right corner of the table, all fall below the 5% level of significance with a single exception, i.e., that between wort nitrogen and salt-soluble nitrogen. This exception results from the fact that barley extract nitrogen is essentially the same fraction as salt-soluble nitrogen. It will be observed that the simple inter-station correlation coefficient between extract nitrogen and salt-soluble nitrogen is 0.966, while that between salt-soluble nitrogen and total nitrogen (1) is 0.851. Thus extract nitrogen is more closely related to salt-soluble nitrogen than the latter is to total nitrogen. This results in a significant partial correlation independent of total nitrogen.

From these correlation studies, it is evident that, within a given variety grown at a number of stations, total nitrogen content is associated with all the nitrogen fractions determined (1) and also with the carbohydrate and nitrogen fractions that contribute to barley extract. The only property measured that does not appear to follow this principle of regularity first enunciated by Bishop (3, 5) is the insoluble cellulose-lignin residue. It is

interesting to note that while the data are not given, the nitrogen content of this residue showed significant correlations with the various nitrogen fractions and that these relations were dependent on common associations with total nitrogen. Thus the only exception to the principle of regularity of composition within varieties appears to be with respect to the structural carbohydrate fraction. Bishop's data (4) also show this same exception. There appears to be no satisfactory explanation of this phenomenon, but it is interesting that the partial correlation studies in Part IX (2) indicate that the structural residue is negatively associated with both starch and barley extract when the effects of total nitrogen are removed. Thus this fraction is anomalous in that it shows regularities with the carbohydrate fractions but not with the nitrogen fractions.

Correlations between Carbohydrate Fractions of Barley and Malting Properties

The simple inter-varietal and inter-station correlation coefficients between the carbohydrate fractions of barley and malt extract, wort nitrogen, steeping time, and malting loss are shown in the upper half of Table II. The corresponding partial correlation coefficients, independent of total nitrogen, are given in the lower half of the table.

TABLE II

SIMPLE AND PARTIAL CORRELATION COEFFICIENTS, INDEPENDENT OF TOTAL NITROGEN, BETWEEN CARBOHYDRATES OF BARLEY AND MALTING PROPERTIES

Barley property	Inter-varietal				Inter-station			
	Malt extract	Wort nitrogen	Steeping time	Malting loss	Malt extract	Wort nitrogen	Steeping time	Malting loss
Simple								
Starch	.854**	−.060	−.174	.359	.967**	−.843**	.793**	.359
Barley extract	.914**	.074	−.311	.428	.973**	−.748**	.814**	.428
Nitrogen in extract	.642*	.833**	−.717**	.856**	−.831**	.672*	−.520	.856**
Cellulose-lignin	−.901**	−.115	.284	−.475	.142	.089	−.367	−.356
Partial								
Starch	.828**	.007	−.089	.374	.625*	−.587	.797**	.218
Barley extract	.899**	.155	−.245	.499	.854**	−.203	.730**	.158
Nitrogen in extract	.617*	.828**	−.700*	.839**	.475	−.138	.233	.247
Cellulose-lignin	−.893**	−.173	.230	−.485	−.596	.546	−.782**	−.195

Inter-varietal Relations

The inter-varietal simple and partial correlation coefficients are of the same order of magnitude, which indicates that the relations depicted in the table are not dependent on relations with total nitrogen. This result was expected since it has been shown in previous studies that the nitrogen content of the

barleys showed no inter-varietal associations with other barley and malt properties. A single exception to this generalization is a significant association between total nitrogen content and the reserve protein hordein, $r = 0.811^{**}$ (1).

It appears from the correlation coefficients between malt extract and the properties listed in the first column of Table II, that the regularities in the carbohydrate constituents of barley (2) are definitely related to the yield of malt extract. The coefficients for starch and barley extract are highly significant and positive, while that for the cellulose-lignin fraction is highly significant and negative.

The only other significant inter-varietal correlation coefficients are those between extract nitrogen and wort nitrogen, steeping time, and malting loss. It appears that these coefficients are significant because extract nitrogen is related to salt-soluble nitrogen and, if partial correlations are computed, independent of this latter property, they all fall below the 5% level of significance.

Inter-station Relations

Within varieties starch and barley extract are positively related to malt extract; extract nitrogen is negatively related; and the cellulose-lignin residue shows no association with malt extract. As would be expected, these relations conform to the regularity principle which exists within varieties. However, it is again evident that the cellulose-lignin residue fails to fit into the regularity. The partial correlation coefficients, independent of total nitrogen, of malt extract with starch and barley extract are significant. This indicates that the regularity principle does not hold rigidly for these relations, since starch and barley extract are more closely related to malt extract than is total nitrogen. It appears that total nitrogen reflects the total carbohydrate of the barley or malt, by difference, but since it fails to reflect the insoluble structural carbohydrate, it is not as closely related to malt extract as direct measures of the reserve carbohydrates.

The significant correlations of wort nitrogen with starch, barley extract, and extract nitrogen reflect the intra-varietal regularities between nitrogen and carbohydrate. The partial coefficients, independent of total nitrogen, again serve to clarify these relations.

Steeping time is positively associated with starch and barley extract and these relations are not dependent on total nitrogen, as shown by the significant partial correlation coefficients. The insoluble residue (cellulose-lignin) is negatively associated with steeping time when the masking effects of total nitrogen are removed by computing the partial coefficient. An increase in the reserve carbohydrates, which contribute to malt extract, results in a longer steeping time or a slower rate of water absorption in the steep. Conversely, an increase in the cellular or structural material is accompanied by a decrease in steeping time or a more rapid absorption. It can be shown that these relations are not entirely dependent on 1000-kernel weight, since

the partial correlation coefficient, independent of 1000-kernel weight, between starch and steeping time just fails to attain the 5% level, while that for barley extract just exceeds it.

Malting loss is positively correlated with extract nitrogen, but the partial coefficient, independent of total nitrogen, indicates that this is merely the reflection of a more fundamental association with total nitrogen.

Regression of Malt Extract on Carbohydrate Fractions of Barley

The correlation coefficients presented in the preceding sections indicate that: starch, barley extract, and cellulose-lignin residue appear to be useful factors for the prediction of malt extract from different varieties grown at the same station; and, starch, barley extract, and total nitrogen appear to be the most suitable factors for prediction within a given variety grown at different stations. These coefficients were computed from varietal and station means, and while they serve to indicate whether the properties are associated with malt extract, they fail to furnish information as to whether these associations are equally close for each variety and each station. To obtain a better understanding of these relations, regressions by stations and varieties were determined and tested for homogeneity. Analyses of residual variance for inter-station or varietal regressions of malt extract on total nitrogen, starch, and barley extract are given in Table III; corresponding analyses for inter-varietal or station regressions on cellulose-lignin residue, starch, and barley extract, appear in Table IV.

TABLE III

ANALYSES OF RESIDUAL VARIANCE FOR VARIETAL REGRESSIONS OF MALT EXTRACT ON TOTAL NITROGEN, STARCH AND BARLEY EXTRACT

Variance due to	D.f.	Mean square		
		Total nitrogen	Starch	Barley extract
Differences among varietal regression coefficients	11	0.3333	0.4275	0.4641
Deviations of varietal means from average regression	11	40.9373**	12.8655**	7.7271**
Residual deviations from individual varietal regressions	120	0.8085	0.6073	0.5589
Deviations from average varietal regression	131	0.7406	0.5998	0.5510

** *Exceeds mean square residual, 1% level of significance.*

To illustrate the discussion and to provide a clearer interpretation of the statistical analyses, the relations between malt extract and barley extract are presented graphically. The scatter diagram, Fig. 1, shows the complete data for these properties, while in Fig. 2 the relation is broken down into its inter-varietal and inter-station components.

The analyses of residual variance, Table III, indicate that the regression coefficients of malt extract on barley extract within varieties do not differ significantly. This means that these regressions, shown in the small scatter

TABLE IV

ANALYSES OF RESIDUAL VARIANCE FOR STATION REGRESSIONS OF MALT EXTRACT ON CELLULOSE-LIGNIN RESIDUE, STARCH AND BARLEY EXTRACT

Variance due to	D.f.	Mean square		
		Cellulose-lignin residue	Starch	Barley extract
Differences among station regression coefficients	11	0.5982	0.9318	0.3408
Deviations of station means from average regression	11	66.8654**	3.5033**	2.9652**
Residual deviations from individual station regressions	120	1.5436	1.4276	1.0378
Deviations from average station regression	131	1.4642	1.3859	0.9507

** *Exceeds mean square residual, 1% level of significance.*

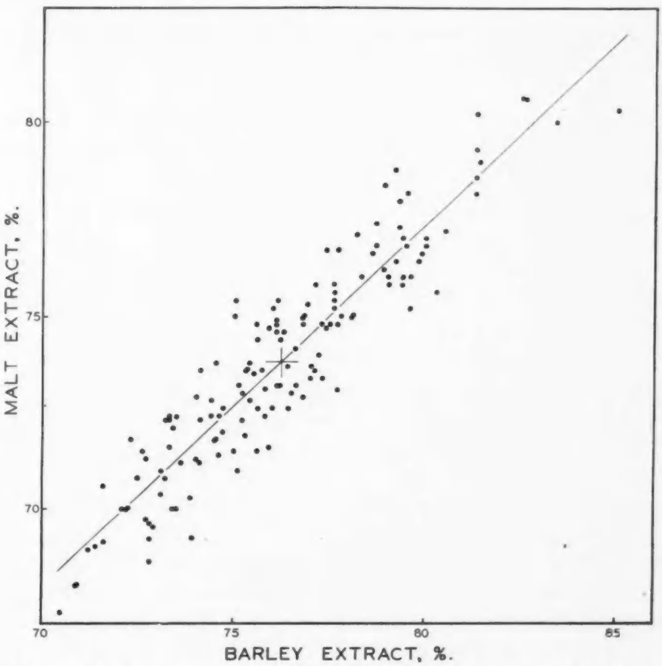

FIG. 1. *Scatter diagram showing the relation between barley and malt extracts.*

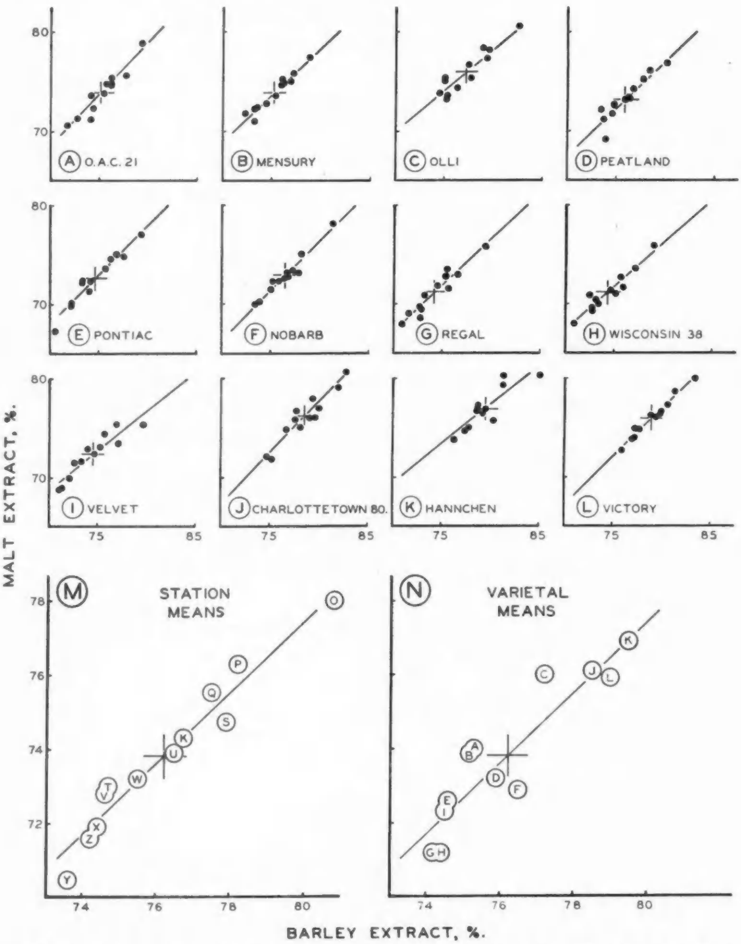

FIG. 2. *Scatter diagrams showing varietal and station relations of barley and malt extracts. The key to stations follows: O, Nappan; P, Fredericton; Q, Ste. Anne de Bellevue; R, Ste. Anne de la Pocatière; S, Lethbridge; T, Winnipeg, U, Brandon; V, Guelph; W, Ottawa; X, Lacombe; Y, Beaverlodge; Z, Gilbert Plains. The key to varieties is given in the small diagrams A to L.*

diagrams *A* to *L* (Fig. 2), may be regarded as a series of parallel lines having the same slope. However, reference to Table III shows that the centroids, which represent varietal means for the properties, deviate significantly from the average varietal regression. If the centroids, marked with a cross in the small diagrams, are superimposed on the average varietal regression, shown in the lower right diagram *N* in Fig. 2, the deviations from the regression line are significant. The analyses of variance indicate that the relations of

malt extract to starch are very similar to those for barley extract. The regressions for nitrogen may also be represented by a series of parallel lines but in this case the slope is negative. The mean square deviation of the individual values from the average regression, shown in the last line of Table III, indicates that malt extract is more closely related to barley extract and starch than to total nitrogen. By means of an "*F*" test it can be shown that the mean square residual for barley extract is significantly lower than that for total nitrogen.

The analyses of residual variance for the inter-varietal regressions of malt extract on barley extract, Table IV, show that these regression coefficients do not differ significantly from station to station. A series of small scatter diagrams for stations would be very similar to those for varieties illustrated in Fig. 2 and the regression lines would all have the same slope. The centroids, or station means, are shown in the lower graph *M*, Fig. 2. From the graph and the analyses in Table IV, it is evident that these centroids deviate significantly from the average station regression. A similar treatment of the data for regression of malt extract on cellulose-lignin residue and starch (Table IV) shows that in each case the regressions can be represented by a series of parallel lines. Those for starch have a positive slope while those for the residue have a negative slope. An "*F*" test applied to the mean square deviations from the average varietal regressions, shown in the last line of Table IV, shows that the relation of malt extract to barley extract is significantly closer than the relation of malt extract to starch or cellulose-lignin residue.

From the preceding discussion it is clear that both within and between varieties the regressions of malt extract on barley extract give the best fit both for the individual values and for variety and station means. Furthermore, the regression coefficients within and between varieties, 0.982 and 0.948 respectively, do not differ significantly and are very close to the total regression coefficient 0.940. These considerations suggest that a fundamental relation exists between barley and malt extract which may be of value for prediction purposes.

Applications to the Prediction of Malt Extract

The relation between barley and malt extracts, Fig. 1, is sufficiently close to suggest that a useful prediction equation might be derived from it. Since this is a linear relation, such an equation would take the form, "$y = a + bx$", when "y" = malt extract, "x" = barley extract, "b" = the regression coefficient, and "a" = the intercept of the regression line and the major ordinate. In brief, the prediction equation becomes the equation of the regression line shown in Fig. 1 and the standard error of prediction is represented by the standard deviation of the individual observations from this line. If the necessary calculations are made, it turns out that the standard error of prediction is $\pm 1.0\%$ in malt extract and the equation is:

$$y = 2.2 + 0.94x.$$

At first sight this equation, based on barley extract alone, does not appear to be quite as accurate as Bishop's "restricted general equation" (5) [$E = 134.7 - 9.0\,N - 2.8I$, in which E = malt extract, N = total nitrogen, and I = insoluble residue]. Both equations have a standard error of ± 1.0, but in Bishop's equation E is given in brewer's pounds per quarter and represents values lying between 91 and 102, whereas in our equation E is given in per cent Plato and represents values lying between 68 and 80. However, since our data show that between varieties the relation of malt extract to barley extract is closer than that of malt extract to insoluble carbohydrate, and that within varieties the relation of malt extract to barley extract is closer than that of malt extract to total nitrogen, the prediction equation based on barley extract alone will give better results with our barleys than an equation similar to Bishop's.

The general equation for the prediction of malt extract from barley extract can be broken down into a number of individual equations, one for each variety and one for each station. These will be the equations for a series of parallel lines passing through the various centroids (i.e., the points shown in Fig. 2, M and N). Since the centroids do not fall on the major regression line, it is evident that the values of the intercept on the major ordinate will differ. Hence a series of varietal and station equations, having the same values of "b" but differing in the constants "a", can be obtained. These equations would serve to define malt extract more accurately but would be of limited usefulness since they apply only to specific varieties or stations. There appears to be no particular point in listing all these equations. However, for comparison with the general equation and since O.A.C. 21 is the recognized standard of malting quality in Canada, the equation for this variety is given below:

$$y = 3.2 + 0.94x,$$

where y = malt extract and x = barley extract.

It is obvious from the preceding sections that it is possible to derive inter-varietal equations from the regression of malt extract on cellulose-lignin residue or starch, and to derive inter-station equations from the regression of malt extract on total nitrogen or starch. However, since these equations will be less accurate than the corresponding equations based on barley extract, it does not appear worth while to consider them in detail.

The relations of barley and malt extract presented in this and earlier papers indicate that barley extract may well represent the total potential malt extract. However, since there is a lack of complete correspondence between these properties as indicated by varietal and station differences in the values of the constant "a", other factors must operate to determine whether the potential extract is realized. Since the enzymes developed during malting serve to liberate the potentially soluble material during mashing, it seems evident that varietal and station differences in enzymatic activity (7) must contribute to the observed differences in the constants "a". This hypothesis can be tested roughly by means of the data shown in Fig. 2 N and data on

the diastatic activity of the varieties, given in Part IV (3). Those varieties (O.A.C. 21, Mensury, and Olli) that give large amounts of malt extract by comparison with barley extract (points *A*, *B*, and *C*, above the line in Fig. 2*N*) should be high in enzymatic activity; whereas those varieties (Nobarb, Regal, and Wisconsin) that give low amounts of malt extract by comparison with barley extract (points *F*, *G*, and *H*, below the line in Fig. 2*N*) should be low in enzymatic activity. In general this proves to be true. The varieties O.A.C. 21, Mensury, and Olli have diastatic activities of 127, 129, and 153, whereas for the varieties Nobarb, Regal, and Wisconsin the values are 100, 85, and 96 respectively. Similar relations hold for proteolytic and starch liquefying activity and furnish additional support for the hypothesis.

If barley extract is considered as potential malt extract, its determination should be of use to plant breeders in selecting varieties that show promise of having superior malting qualities from a number of hybrid lines of equal agronomic quality. It is evident that, unless a new variety has a barley extract as high or higher than that of the standard malting variety there is little or no chance of its malt extract yield exceeding that of the standard. However, it should be noted that if lines are selected on this basis, some of them may not produce malts of high extract yield because of deficiencies in enzymatic activities. Thus it will only be profitable to apply a prediction based on barley extract to hybrids resulting from a cross in which at least one of the parents is known to be of reasonably high enzymatic activity. Moreover, owing to the differential effect of environment on varieties, if the hybrid material is grown at one station only, local conditions may tend to conceal average varietal differences in extract yield which might become evident if the lines were grown at a number of stations. In spite of these limitations, this method of selection appears to have a definite value. Determinations of barley extract can be made quite rapidly (12 per day per man) and require only a small amount of grain. It should therefore be possible to apply it on a much wider scale and at an earlier stage in the process of selection than is possible with laboratory malting test.

The foregoing discussion serves to illustrate the development and possible utility of prediction equations based on the relations between malt extract and individual barley properties. It is also possible to derive equations involving the simultaneous relations of malt extract and two or more barley properties (e.g., 3, 4). Equations of this type, which permit more accurate prediction, will be discussed in the next paper in this series.

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HYBRIDIZATION OF *TRITICUM* AND *AGROPYRON*

VI. INDUCED FERTILITY IN VERNAL EMMER × *A. GLAUCUM*[1]

BY F. H. PETO[2] AND J. W. BOYES[2]

Abstract

Colchicine treatments of the seed of a sterile F_1 hybrid of Vernal emmer × *A. glaucum* ($2n = 35$) induced fertility through chromosome doubling. The F_2 plants were exceptionally vigorous, with abundant leafy foliage, and spikes producing an average of 48 seeds each, of which 83% were viable. These amphidiploid plants appear to be perennial, but so far have not been tested for winter hardiness.

The establishment of amphidiploid sectors of sufficient size to make possible the production of seed depended on the successful competition of the amphidiploid with the undoubled tissue. Different hybrids varied greatly in this respect. A relation existed between growth vigour of amphidiploid sectors, or plants, and increase in stomatal size on chromosome doubling.

Eight of eleven F_2 plants possessed 70 chromosomes and the remaining three, 64, 68, and 69. Meiotic studies on five of these plants revealed the formation of 27 to 31 bivalents, 6 to 9 univalents, and occasional trivalents and quadrivalents. Anthers of the amphidiploids dehisced abundant pollen, 94% of which was good, whereas anthers of the undoubled F_1 were unable to dehisce and contained only 2% good pollen.

Introduction

The problem of inducing fertility in sterile intergeneric hybrids in Gramineae by doubling the chromosome number has been receiving considerable attention in recent years. Prior to the discovery of the value of colchicine for this purpose, temperature treatments were found to be moderately successful. The senior author (6) in 1937 doubled the chromosome number in the F_1 of *T. vulgare* var. Kharkov ($2n = 42$) × *A. glaucum* ($2n = 42$) through the application of alternating high and low temperature treatments on the early zygotic division. The resulting 84-chromosome plant lacked vigour and failed to produce spikes prior to publication of the results in December 1938. However, two spikes formed a few months later on clones growing in the greenhouse. These spikes dehisced an abundance of good pollen and a number of plump wheat-like seeds were formed.

The first successful attempt to use colchicine to induce fertility in *Triticum-Agropyron* hybrids was reported in 1939 by Raw (7) who immersed clones of the F_1 of *T. vulgare* × *A. intermedium* in a 0.2% colchicine solution for 10 hr. and obtained one plant that was fully fertile. Sears (8) subsequently reported doubling the chromosome number in sterile hybrids of *T. monococcum* × *Aegilops uniaristata*, *Ae. caudata* × *Ae. umbellulata*, and *Ae. spleltoides* × *Ae. umbellulata* by immersing the germinating seeds in 0.05% solution for

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Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. This contribution forms part of a co-operative investigation on the hybridization of Triticum and Agropyron, undertaken by the Dominion Experimental Farms and the National Research Council of Canada. N.R.C. No. 910.

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24 hr. A high degree of fertility was noted in the amphidiploid sectors of these hybrids.

The present paper gives the results of colchicine treatments on ungerminated F_1 seeds of *Triticum-A. glaucum* hybrids.

Results of Colchicine Treatments

Colchicine treatments were applied in October 1938 to three-year-old seed from crosses of Vernal emmer, Lutescens, and Mindum with *A. glaucum*, and to fresh seed from crosses of Lutescens, Ruby, C.A.N. 1835, and Garnet with *A. glaucum*. The method of seed treatment was similar to that used by Myers (4), who had kindly outlined his procedure prior to publication.

The seed treatments of the various hybrids are given in Table I. The best results were obtained in treatments with 0.2% colchicine for 24 hr. duration. Sixteen seedlings out of 109 appeared to have external symptoms of chromosome doubling. The stomatal sizes of three of these seedlings were determined. At this time Mindum × *A. glaucum* 5-2 appeared to be completely amphidiploid since all the stomata examined in the fourth leaf were abnormally large. About three-quarters of the epidermal leaf tissue of Lutescens × *A. glaucum* 4-1 also appeared to have the doubled complement. In Mindum × *A. glaucum* 5-3, 100 stomata were measured in normal as well as in amphidiploid sectors of the fourth seedling leaf. In the normal sectors, the average length of the guard cells was 63.3 μ, the width 34.6μ, and the

TABLE I

COLCHICINE TREATMENTS OF *Triticum-A. glaucum* HYBRID SEED

Treat. No.	Cross	Colchicine treatment (24 hr.)	No. seeds treated	Total no. seedlings	Seedlings with external symptoms of chr. doubling
1	Vernal × *A. glaucum*	Check	8	8	0
2	Vernal × *A. glaucum*	0.2%	15	6	0
3	Vernal × *A. glaucum*	0.4%	15	2	0
4	Lutescens × *A. glaucum*	0.2%	17	6	1*
5	Mindum × *A. glaucum*	0.2%	14	7	1, 2*, 3*
6	Lutescens 0.62 × *A. glaucum*	0.1%	10	5	7
7	Lutescens 0.62 × *A. glaucum*	0.2%	20	13	12, 13, 14
8	Lutescens 0.62 × *A. glaucum*	0.4%	15	11	25
9	Lutescens 0.329 × *A. glaucum*	0.1%	10	10	36
10	Lutescens 0.329 × *A. glaucum*	0.2%	20	10	46, 47
11	Lutescens 0.329 × *A. glaucum*	0.4%	20	8	0
12	Ruby × *A. glaucum*	0.2%	20	9	1
13	C.A.N. 1835 × *A. glaucum*	0.2%	15	10	1, 2
14	Garnet × *A. glaucum*	0.2%	10	3	0
15	Kharkov × *A. glaucum*	0.2%	28	1	1
	Total		237	119	16

* *Stomatal measurements on these seedlings indicated the presence of sectors possessing doubled chromosome number.*

product 2,176 sq. μ. In the amphidiploid sectors, the average length was 80μ, the width 40.5μ, and the product $3{,}250\mu$. The mean product of the length and width in the amphidiploid sectors is 49.4% larger than in the normal sectors. There is no doubt that these clear-cut differences between stomatal dimensions in the same region of the leaf are the result of chromosome doubling.

Epidermal strips were taken from leaves of selected plants at three or four stages during development. It was not possible to strip the epidermis from the entire lower surface of a leaf, particularly in the seedling stages, consequently all the tissue in any leaf could not be examined. This condition improved as the plants grew older and wider strips could be obtained. Measurements of stomata in these strips made it possible to detect tissue with the double chromosome number and to observe its survival during development.

The data obtained from four plants with doubled sectors are given in Table II. In the Mindum × *A. glaucum* hybrids, the doubled (10x) tissue appeared to be able to compete successfully with the normal in the young seedlings, but was rapidly eliminated during later development. In the Lutescens × *A. glaucum* hybrids, the tissue with the doubled (12x) complement appeared to be able to compete more successfully with the undoubled cells throughout development. These data indicate that the survival throughout development of the tissue with the doubled chromosome number differs greatly in the two crosses. Apparently, competition between doubled and undoubled tissue determines the relative amounts of these tissues present in the mature plant.

In addition to stomatal observations it was possible to detect broad strips of doubled tissue by the external appearance of the leaf in certain hybrids, the doubled portions being coarser with crooked veins. In the Lutescens-*A. glaucum* plants at maturity it was possible to see the arrangement of the doubled and undoubled tissue in all the living leaves on the plant. For example, in plant 8-25, half of each blade was completely doubled and the other half was normal, the mid-rib being the dividing line. It was found that the doubled tissue in successive leaves was found alternately on the right and left side of the mid-rib. The fact that the effect was not independent in alternate leaves indicated that the central axis had been similarly affected. Consequently it was expected that half the spike might have the doubled constitution. In spite of these expectations, this particular spike at maturity was completely sterile, indicating that the central axis was not permanently affected.

None of the plants that showed symptoms of chromosome doubling during development produced fertile spikes. However, one Vernal emmer × *A. glaucum* plant (2-12) that failed to show external symptoms of doubling produced nine spikes, one of which was completely fertile, producing 21 seeds. This result emphasizes the fallacy of depending entirely on external symptoms

TABLE II

STOMATAL MEASUREMENTS ON HYBRID PLANTS TAKEN AT DIFFERENT STAGES OF DEVELOPMENT

Cross	Plant No.	Age plants (days)	Stage of development	Epidermis with normal sized stomata: No. of stomatal rows	Epidermis with normal sized stomata: Av. stomatal dimensions, μ: Length	Epidermis with normal sized stomata: Av. stomatal dimensions, μ: Width	Epidermis with abnormally large stomata: No. of stomatal rows	Epidermis with abnormally large stomata: Av. stomatal dimensions, μ: Length	Epidermis with abnormally large stomata: Av. stomatal dimensions, μ: Width	General observations
Mindum × *A. glaucum*	5–2	50	4th leaf	0	—	—	9	82.5	39.9	Leaf apparently completely 10x constitution.
		103	6th leaf	6	63.8	31.9	5	82.5	37.2	Leaf ½ 10x, alternating strips of 5x and 10x tissue.
		103	7th leaf	7	65.2	29.3	4	75.8	37.2	Leaf ⅓ 10x, alternating strips of 5x and 10x tissue.
		147	11th leaf	39	57.2	26.6	0	—	—	Leaf completely of 5x constitution.
Mindum × *A. glaucum*	5-3	50	3rd leaf	3	63.8	31.9	2	85.1	39.9	Leaf app. ½ 10x, alternating strips of 5x and 10x tissue.
		50	4th leaf	6	63.8	34.6	7	79.8	39.9	As 3rd leaf.
		103	7th leaf	22	66.5	30.6	0	—	—	Leaf completely of 5x constitution.
Lutescens × *A. glaucum*	4–1	53	4th leaf	4	61.2	35.9	15	86.4	43.9	Leaf app. ¾ 12x, narrow strips of 6x tissue between wide strips of 12x tissue.
		103	7th leaf	4	66.5	27.9	6	83.8	43.9	Leaf app. ½ 12x.
		147	Shot blade	20	58.5	29.3	16	73.1	39.9	Leaf app. ½ 12x. One side of midrib pure 12x tissue.
Lutescens × *A. glaucum*	8–25	96	6th leaf	9	67.8	35.9	3	91.8	43.9	Leaf app. ¼ 12x, narrow strips of 12x dispersed between 6x tissue.
		140	Shot blade	34	54.5	29.3	3	75.8	39.9	Leaf app. ½ 12x, narrow strips of 12x dispersed between 6x tissue.

for the detection of amphidiploid tissue, since it may be entirely normal in appearance, and increase in cell size would only be apparent on microscopic examination.

Development and Fertility of Amphidiploid Vernal emmer × *A. glaucum*

The seed produced on the fertile F_1 spike was compared with the two parents. The weight per 1000 kernels in grams for Vernal emmer is 34.2, for the F_1 16.6, and for *A. glaucum* is 5.7 (Fig. 2). The amphidiploid hybrid seed is thus about three times as heavy as the seed of the grass parent and one-half that of the wheat parent. Twelve seeds were sown in the spring of 1939 and all germinated. Chromosome numbers were determined for 11 of the plants, either by root tip or p.m.c. examination. Eight plants had 70 chromosomes, a number, of course, exactly double that of the normal F_1, and the three other plants had 64, 68, and 69 chromosomes.

Twelve F_2 seeds were sown on May 16, 1939, and transplanted to the garden early in June, where they grew vigorously.* They produced an abundance of leafy foliage of slightly coarser texture than that of the undoubled F_1, but as fine or finer than that of most *Triticum-A. glaucum* hybrids. By late autumn flowering spikes were developing freely, and a total of 503 seeds had developed by mid-October when it was necessary to harvest the material. Detailed data on the fertility and germinability for nine of the 11 spikes harvested are given in Table III. In spite of the lateness of maturity a remarkably good seed set was obtained. An average of 48 seeds per spike was produced, which is believed to be in excess of *A. glaucum* and would compare favourably with Vernal emmer. Counts were made on the number of well developed florets per spike and the percentage fertility was based on this. An average of 77% of the florets produced seed, two of the plants (2-12-1 and 2-12-7) being definitely superior in this regard. The fourth

TABLE III

FERTILITY OF THE F_2 AND VIABILITY OF F_3 SEED OF AMPHIDIPLOID PLANTS OF VERNAL EMMER × *A. glaucum*

Plant No.	Number spikes examined	Number spikelets	Number florets	Number seeds	Fertile florets, %	Germination, %
2-12-1	5	104	321	260	81.0	87.2
2-12-5	1	19	45	27	60.0	88.9
2-12-7	2	42	142	115	81.0	75.2
2-12-9	1	22	61	34	55.7	88.2
Total	9	187	569	436		
Average		21 per spike	63.2 per spike	48.4 per spike	76.6	83.5

* *In the autumn of 1939, 30 F_2 and 254 F_3 seeds of the Vernal-A. glaucum amphidiploid were supplied to the Division of Forage Plants, Central Experimental Farm, Ottawa. The resulting plants showed considerable promise, as reported in Magazine Digest, May, 1940. An additional 6,400 seeds were supplied in April, 1940.*

floret was fertile in 43% of the spikelets of 2-12-7 and in 18% of the spikelets of 2-12-1, whereas neither of the remaining two plants (2-12-5 and 2-12-9) had any fertile fourth florets. However, undue emphasis should not be placed on the relative seed production in the year of seeding. In the second year the seed should ripen much earlier and under these more favourable conditions it is expected that fertility, plumpness of seed, and total yield of all the plants will be greatly increased.

The germination data in Table III show that the viability of the seed is satisfactory, especially since some of it was immature when harvested.

The colchicine-treated F_1 plant (2-12) that produced the fertile spike was transplanted to the garden in the spring of 1939 and it again produced one fertile spike (21 seeds) and 20 sterile spikes. The plant was cloned in the autumn into 16 portions in an endeavour to isolate the amphidiploid sector.

A comparison of stomatal size in doubled and undoubled leaf tissue of Vernal × *A. glaucum* is shown in Table IV and Figs. 3 and 4. There is a striking increase in the length and width of the guard cells and the area index of the 70-chromosome plant is 109% greater than that of the 35-chromosome plant.

TABLE IV

COMPARISON OF STOMATAL SIZE OF DOUBLED AND UNDOUBLED LEAF TISSUE

Hybrid	Plant No.	Chr. no. ($2n$)	No. stomata examined	Mean length (μ)	Mean width (μ)	Area index ($L \times W$)
Mindum × *A. glaucum* (undoubled sector)	3	35	100	63.3	34.6	2,176
Mindum × *A. glaucum* (doubled sector)	3	70	100	80.0	40.5	3,250
Vernal × *A. glaucum* (undoubled F_1)	2–12	35	100	53.1	27.8	1,476
Vernal × *A. glaucum* (doubled F_2)	2–12–8	70	100	86.0	35.8	3,083

The data in Table V indicate a relation between growth vigour of amphidiploid sectors or plants, and increase in stomatal size on chromosome doubling. From this it is concluded that the more vigorous the amphidiploid tissue the greater is the increase in stomatal size on chromosome doubling. This relation is most obvious in the comparison of the Kharkov × *A. glaucum* with the Vernal × *A. glaucum*, but is also indicated by the relative ability of the doubled tissue to compete successfully with the undoubled tissue in the other two crosses.

The amphidiploid Vernal × *A. glaucum* material appears to have excellent agronomic possibilities, although little should be said on this point until extensive field trials have been completed. However, it has one possible fault in that the rachis shatters on threshing like the Vernal parent. The seeds are not held tightly in the glumes and close threshing may free them.

TABLE V

RELATION OF VIGOUR OF AMPHIDIPLOID SECTORS OR PLANTS TO INCREASE IN STOMATAL SIZE ON CHROMOSOME DOUBLING

Hybrids	Increase in area index on chr. doubling, %	Observations on vigour
Kharkov × *A. glaucum*	36	Amphidiploid plants less vigorous than sterile F_1.
Mindum × *A. glaucum*	49	Doubled tissue (10x) unable to compete successfully with undoubled tissue.
Lutescens × *A. glaucum*	78	Doubled tissue (12x) able to compete successfully with undoubled tissue.
Vernal × *A. glaucum*	109	Amphidiploid plants more vigorous than sterile F_1.

If this is not possible, it will be necessary to sow the whole spikelet, which will be wasteful as it may contain up to four seeds. It should be possible to breed away from this spike character by crossing with other 70-chromosome amphidiploids. In an effort to produce other amphidiploids for this purpose, seed and seedlings of 12 additional *Triticum-A. glaucum* hybrids have recently been treated with colchicine.

The excellent leafiness, tillering capacity, and vigour of the amphidiploid are illustrated in a photograph (Fig. 1) of plant 2-12-1 taken on November 2, 1939. The height is indicated by the yard stick. The plants show definite indication of being perennial as their clones are growing vigorously. Winter hardiness has not yet been tested, but the sterile F_1 is known to be reasonably winter hardy in Ottawa and the more vigorous amphidiploid derivative is not likely to be different in this respect.

Cytological and Pollen Observations

It was previously reported (5) that the sterile F_1 of Vernal emmer × *A. glaucum* possessed an average of 20.4 univalents, 6.2 bivalents, and 0.8 trivalents per nucleus. The low degree of homology between the *Triticum* and *Agropyron* chromosomes undoubtedly accounts for the complete sterility of the undoubled F_1 generation. Four meiotic stages in this material are illustrated in Figs. 5–8. The univalents lag at first anaphase and divide equationally. In the second division the half univalents wander at random and those that do not happen to be included in the tetrad nuclei form micronuclei which subsequently degenerate.

On doubling the chromosome number in the F_1 of Vernal emmer × *A. glaucum*, each chromosome should have a completely homologous partner and 35 bivalents would be expected. These expectations were not realized, since in the five F_2 plants examined (Table VI) averages of from 27 to 30 bivalents were found. The failure of all the chromosomes to pair cannot be attributed to lack of homology but rather to the lack of opportunity for

TABLE VI

SUMMARY OF MEIOTIC CHROMOSOMAL ASSOCIATIONS FOR F_2 VERNAL EMMER × *A. glaucum* AMPHIDIPLOIDS

Plant No.	No. of cells	Associations of				P.M.C. count
		I	II	III	IV	
2-12-1	20	7.3	30.7	0.1		69
2-12-2	20	8.7	27.55		0.05	64
2-12-5	20	8.0	30.6	0.2	0.05	70
2-12-7	20	6.0	30.9		0.05	68
2-12-8	20	8.65	30.25	0.15	0.10	70

all of the homologous chromosomes to pair during the zygotene stage, at which time homology presumably results in pairing. It appears reasonable to suppose, as also suggested by Kostoff (2), that the difficulty of pairing would be increased on doubling of the chromosome number. It follows that there must be some limit in chromosome number beyond which zygotene pairing becomes difficult in spite of perfect homology. In the Vernal × *A. glaucum* amphidiploid nucleus possessing 70 large chromosomes, either the duration of the zygotene stage or the pairing attraction may be inadequate to overcome the difficulties caused by the increase in spatial distribution of the homologous chromosomes.

Investigations of Berg and Oehler (1) and Müntzing (3) indicate that genetic factors influence pairing conditions in *Triticale*. Müntzing suggests that the main cause of the meiotic lability in *Triticale* is an automatic inbreeding degeneration of the rye component. His explanation would not apply to the situation in the Vernal × *A. glaucum* amphidiploid in which both parents are largely self-fertilized and the material studied has been inbred for only one generation. However, genetic factors capable of causing variation in pairing conditions possibly exist in this material, and forms in which a high percentage of the chromosomes form bivalents may eventually be obtained by selection.

It has not been possible to obtain p.m.c. material from the amphidiploid sector of the F_1 plant, but it can be assumed that similar meiotic irregularities existed as indicated by the variable chromosome numbers in the F_2 noted above.

In spite of the differences in the chromosome number of the F_2 plants examined cytologically, there were only small differences in the frequency of the various configurations both within and between plants. For example, two plants had 26-33 bivalents and others had 27-33, 28-33, and 25-30 bivalents per nucleus. The p.m.c. material of plant 2-12-2, which had the lowest mean number of bivalents (27.5) and the lowest range (25-30), was collected on October 13, at least two weeks later than any other material examined. Thus it is probable that the cool weather conditions existing at the time this material was collected may have influenced pairing.

The number of multivalent configurations was much lower than the theoretical expectation on the basis of pairing in the undoubled F_1. Since an average of six bivalents per nucleus was found in the sterile F_1, it should be possible for six quadrivalents per nucleus to appear in the doubled F_2. Actually only 9 trivalents and 5 quadrivalents were observed in the hundred nuclei examined, which indicates that the chromosomes pairing in the undoubled F_1 were only partially homologous. It is also of interest to note that 10 out of the 14 multivalent configurations occurred in the two 70-chromosome plants.

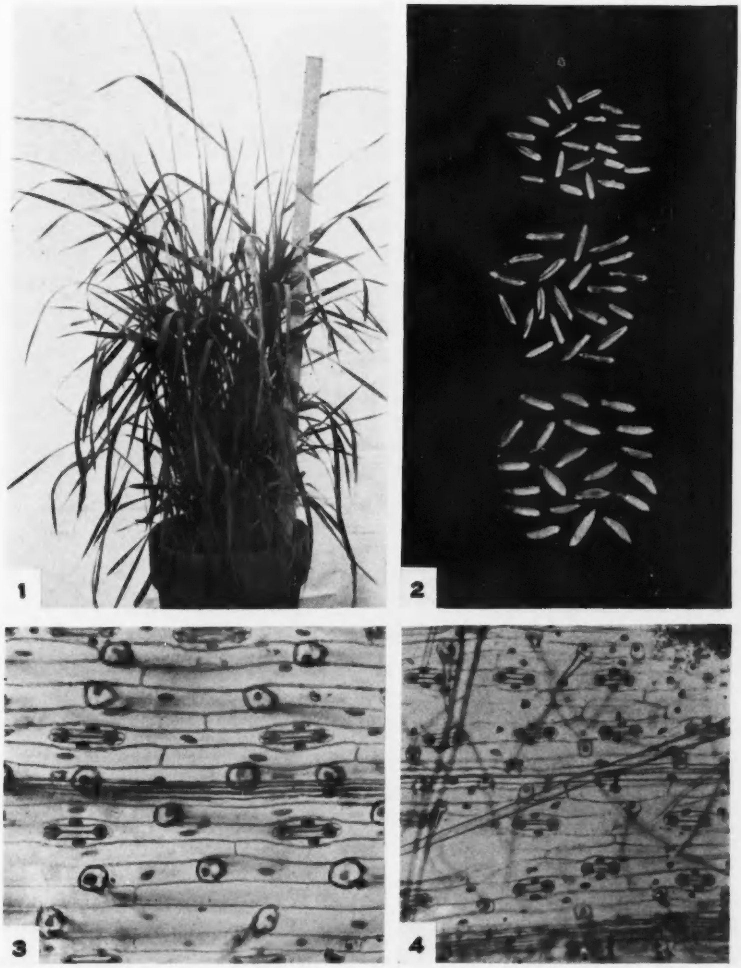
Meiosis in the fertile amphidiploid is shown in Figs. 9–16. The range in bivalent formation is illustrated by Figs. 9 and 10. In the former there are 10 univalents and 27 bivalents, while in the latter there are two univalents and 33 bivalents. The behaviour of the univalents is similar to that found in the undoubled F_1. The lagging and splitting of univalents in the first division is illustrated in Figs. 11–13. The movement at random of the half univalents in the second division is shown in Fig. 14 and the resulting micronuclei in the tetrads are shown in Fig. 15. The average number of micronuclëi per tetrad in four plants is recorded in Table VII. The four plants were very similar in this respect and averaged approximately four micronuclei per tetrad. In spite of the relatively frequent occurrence of micronuclei, the pollen appeared to be very good. (Fig. 16).

TABLE VII

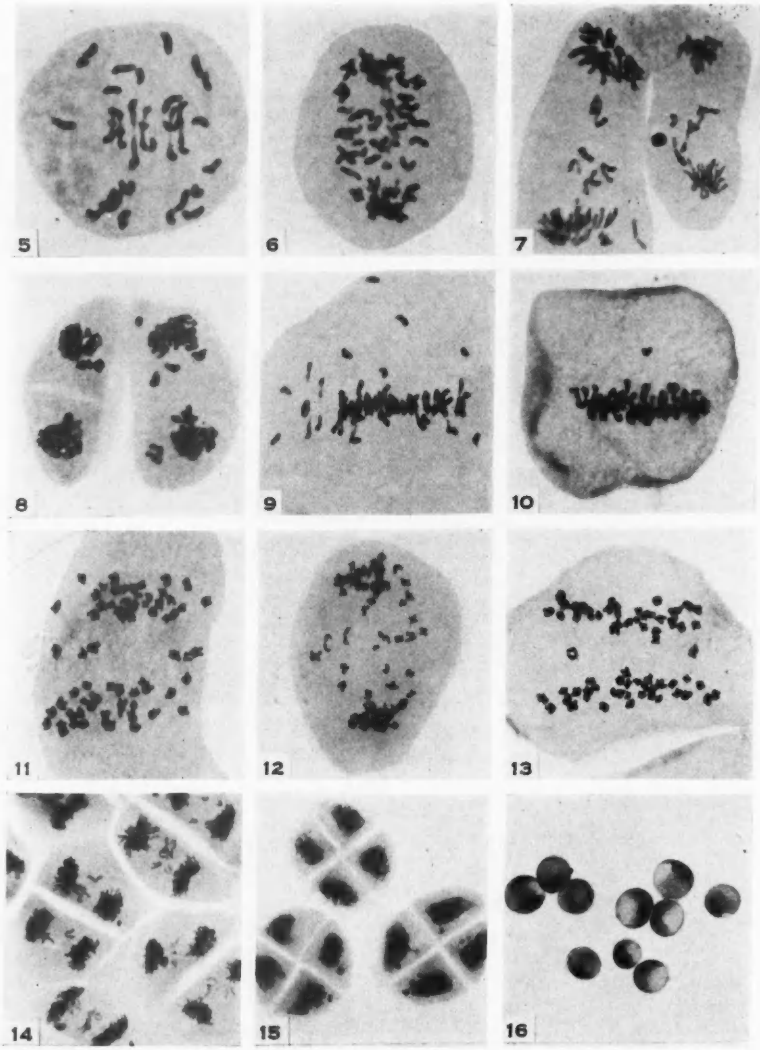
MICRONUCLEI IN TETRADS OF F_2 AMPHIDIPLOID VERNAL EMMER × *A. glaucum* PLANTS

Plant No.	No. tetrads	No. micronuclei	Average no. per tetrad
2-12-1	40	144	3.6
2-12-5	40	149	3.7
2-12-7	40	186	4.6
2-12-8	40	173	4.3
Total	160	652	
Average			4.08

A detailed study of the pollen of five F_2 plants and the undoubled sector of the F_1 plant was made. The pollen was graded on the basis of the number of nuclei present and as to whether the quantity of the cytoplasm was normal, reduced, slight or none. The individual amphidiploid plants were very similar in that a high proportion (92 to 97%) of their pollen grains had three nuclei and the normal amount of cytoplasm. Consequently only the summary of all the plants is shown in Table VIII. A total of 472 out of 500 amphidiploid pollen grains (94%) possessed three nuclei and the normal amount of cytoplasm, whereas less than 2% of the pollen grains of the undoubled F_1 could be considered normal. The proportion of good pollen



FIGS. 1 – 4. FIG. 1. *F_2 amphidiploid Vernal emmer × A. glaucum 2-12-1.* FIG. 2. *Seeds: above, A. glaucum; below, Vernal emmer; centre, amphidiploid F_1 Vernal emmer × A. glaucum.* FIG. 3. *Stomata of F_2 amphidiploid Vernal emmer × A. glaucum 2-12-8.* FIG. 4. *Stomata of the sterile F_1 Vernal emmer × A. glaucum. Magnification of Figs. 3 and 4, ×135.*



FIGS. 5 – 16. FIGS. 5 – 8. *Vernal emmer* × *A. glaucum sterile* F_1. FIG. 5. *First metaphase 23xI, 6xII.* FIG. 6. *First anaphase showing numerous univalents lagging and dividing at the equatorial plate.* FIG. 7. *Second division showing lagging half-univalents.* FIG. 8. *Tetrad with micronuclei.* FIGS. 9 – 16. *Vernal emmer* × *A. glaucum fertile* F_2 *amphidiploids.* FIG. 9. *First metaphase of 2-12-2, 10xI and 27xII.* FIG. 10. *First metaphase of 2-12-7 with 2xI and 33xII.* FIG. 11. *First anaphase of 2-12-8* $(2n = 70)$. FIG. 12. *Late first anaphase of 2-12-2, dividing univalents.* FIG. 13. *First anaphase 2-12-7* $(2n = 68)$. FIG. 14. *Lagging half-univalents in second anaphases 2-12-8.* FIG. 15. *Tetrads with micronuclei 2-12-7.* FIG. 16. *Pollen 2-12-1. Aceto-carmine smear preparations. Magnification of Figs. 5 – 8 is* ×*680, of Figs. 9 – 15 is* ×*410, of Fig. 16 is* ×*85.*

TABLE VIII

SUMMARY OF POLLEN OBSERVATIONS ON AMPHIDIPLOID F_2 AND UNDOUBLED F_1 OF VERNAL × *A. glaucum*

Plant No.	Cytoplasm	Number of nuclei				Total	Per cent
		3	2	1	0		
2-12-1, 2-12-5, 2-12-7, 2-12-9, 2-12-10	Normal	472	2	0	0	474	95.8
	Reduced	8	1	0	1	10	2.0
	Slight	1	3	0	2	6	1.2
	None	0	0	0	10	10	2.0
	Total	481	6	0	13	500	
	Per cent	96.2	1.2	0.0	2.6		100
Undoubled F_1 (2-12)	Normal	4	9	4	0	17	6.8
	Reduced	0	2	31	0	33	13.2
	Slight	2	3	75	19	99	39.6
	None	0	0	0	101	101	40.4
	Total	6	14	110	120	250	
	Per cent	2.4	5.6	44.0	48		100

in the undoubled F_1 was so low that dehiscence never occurred, while the F_2 plants dehisced an abundance of good pollen.

Acknowledgments

The authors are greatly indebted to Dr. T. M. Stevenson, Dr. J. M. Armstrong, and Mr. A. McLennan of the Division of Forage Plants, Central Experimental Farm, Ottawa, for supplying hybrid seed, and for generous cooperation at all times.

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STUDIES ON BROWNING ROOT ROT OF CEREALS

VI. FURTHER CONTRIBUTIONS ON THE EFFECTS OF VARIOUS SOIL AMENDMENTS ON THE INCIDENCE OF THE DISEASE IN WHEAT[1]

By T. C. Vanterpool[2]

Abstract

Further work has substantiated earlier findings that phosphatic fertilizers and farm manure will give adequate control of *Pythium* root rot of wheat in infested prairie soils. The improvement in growth resulting from these amendments is considered to be due to the production of a larger number of quicker growing roots which lessens the chances for infection and leaves more roots healthy, though the same percentage may be affected as in diseased plants showing severe leaf discolorations. Experiments have failed to indicate that the phosphatic materials increase resistance appreciably. Nitrogenous materials when applied singly had virtually no effect on growth, but once ample phosphorus was added, further nitrogen applications gave substantially greater increases than phosphate alone. Phosphorus is apparently the chief limiting element. No difference was found in preliminary tests in the phosphate-fixing power of browning and normal soils. Typical browning soils responded irregularly to small applications of boron, copper, manganese, or zinc, but were not found to be seriously lacking in these elements. Moderate benefits resulted from heavy applications of gypsum and of sulphur. Browning soil was found also to be deficient in phosphate for non-cereals such as alfalfa, buckwheat, carrots, flax, lettuce, and sweet clover. These crops were not attacked by the *Pythium* spp. pathogenic to cereals. Consequently the poor growth of the non-cereals in browning soil appears to be due to nutrient deficiencies, while the poor growth of cereals is due to both root-destroying fungi and nutrient deficiencies. In both instances phosphorus is probably the chief limiting element. Ground cereal straw, sweet clover hay, and weed hay amendments gave moderate increases in the growth of wheat. No consistent differences were found in the carbon-nitrogen ratios of browning and normal soils. The results as a whole suggest that two of the most practicable means of meeting the browning root-rot situation are, firstly, to supply supplemental nutrients in the form of artificial fertilizers, and secondly, to add organic residues or farm manure regularly to fields subject to the disease.

Introduction

It has been found difficult to reduce and impossible to destroy under field conditions the species of *Pythium* commonly found attacking wheat roots on the Canadian prairies. Thus both *Pythium arrhenomanes* Drechsl. and *P. tardicrescens* Vanterpool, the two species most frequently isolated from browning root-rot lesions in recent years, have repeatedly been obtained from the roots of wheat seedlings grown in soils that had been in clean fallow for ten years or more. It is not known whether the resting oospores of these species of *Pythium* are capable of retaining their vitality for that length of time in the soil, or whether these fungi can live saprophytically as members of the natural soil microflora. Their poor competitive abilities with other

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micro-organisms in artificial culture does not lend support to the latter view. According to Carpenter (5) the species of *Pythium* causing root rot of sugar-cane, which is also pathogenic on wheat, may grow saprophytically in the soil, but it is not a vigorous competitor with the strictly saprophytic soil fungi. Recontamination of fallowed land by wind-blown oospores during dust storms probably occurs quite frequently, as is indicated by the finding of the pathogens in the roots of wheat seedlings grown in soil collected from deep drifts some distance from cultivated fields, and in soil from the eaves-troughs of city houses in Saskatoon.

As it has not been feasible to starve or destroy the root-infecting fungi by any practicable farm operations, studies on the improvement of soil conditions and other factors which might (i) lead to the development of more vigorous plants, and (ii) tend to check the activity phase of the parasites, were accordingly undertaken. Some results on the effects of various soil amendments on the root rot have been reported in Part IV (20) of this series. The earlier experiments have been repeated and extended and the results used as a basis for this paper.

Methods Used in the Greenhouse Investigations

The most feasible method of studying browning root rot experimentally is to use naturally-infested field soil and conduct the experiments under controlled conditions in the greenhouse, or out-of-doors.

The soil is collected in June from severely attacked fields; the composite of *Pythium*-infested soil from diseased areas forms the stock of browning soil, and the composite from healthy areas forms the stock of normal soil. Quantities of these soils are taken as required and filled into six-inch containers. Artificial fertilizer applications are incorporated into the soil at a distance of 1 in. below to 1 in. above seed level, while the organic amendments are mixed through the top 4 in. of soil. Marquis seed of good quality is sown at a depth of 2 to $2\frac{1}{2}$ in. to permit the development of crown roots at some distance from the seminal roots so as to facilitate counting. Twelve seeds are sown to each six-inch pot or one-gallon crock and later thinned out to ten; this eliminates any overcrowding effects up to the five- to six-weeks stage when browning symptoms are most conspicuous. The pots are randomized and rerandomized from time to time during the course of the experiment. Supplemental overhead illumination is necessary during the winter months. About once a week watering is done from below by placing the containers in water to a depth of 1 in. until the surface soil becomes moist; at all other times watering is done from above. In studies on comparative methods of watering, the one just outlined has been found most suitable for experiments on root rots. Oven-dry weights refer to the weights of the tops. Root-lesion counts are confined to the crown roots because of the impracticability of estimating visually the amount of disease on the fine seminal root system.

Commercial Fertilizer Amendments and Wheat Response

In experiments reported previously, Vanterpool (20, Part IV) showed that phosphatic amendments permitted the development of normal wheat plants in soil naturally infested with ***Pythium***, while inorganic nitrogenous fertilizers usually had no effect. In the following experiments, materials used previously have been tested further and new ones included.

1. Various Fertilizers and Fertilizer Combinations

Experiment 1. The phosphatic fertilizers were applied at rates equivalent to 100 lb. of phosphorus pentoxide per acre, the nitrogenous fertilizers at 96 lb. of nitrogen per acre, and the potassic fertilizers at 98 lb. of potassium oxide per acre. The materials were mixed into the soil at seed level, as previously described, at the time of planting. The seedlings were harvested after six weeks, by which time browning symptoms were well advanced in the controls.

In Experiment 1, Table I, phosphorus applied singly as triple superphosphate has given only a moderate increase, though much larger increases are usual, as will be seen from the results in Experiment 2, Table I, and from

TABLE I

THE EFFECT OF FERTILIZERS ON THE GROWTH OF WHEAT SEEDLINGS IN NATURALLY INFESTED FIELD SOIL

Treatment	Height, cm.	Seminal roots per plant	Crown roots			Dried plants	
			Total no.	No. healthy	Diseased, %	Weight, gm.	Increase, %
*Experiment 1**							
None—control	44	—	21	13	38.1	6.7	—
Triple superphosphate (1-43)	48	—	59	35	40.6	9.2	37.3
Ammonium phosphate (11-48)	52	—	73	44	39.7	13.5	101.5
Ammonium phosphate (16-20)	56	—	80	40	50.0	15.4	129.8
Ammonium sulphate	48	—	21	9	42.8	8.1	20.9
Ammonium nitrate	45	—	17	7	58.8	8.0	19.4
Calcium nitrate	44	—	22	10	54.5	7.0	4.5
Sodium nitrate	45	—	15	6	60.0	7.1	5.9
Potassium nitrate	47	—	23	18	21.7	7.7	14.9
Urea	47	—	23	16	30.4	8.1	20.9
Cyanamide	46	—	28	16	42.8	7.1	5.9
Potassium sulphate	44	—	21	13	38.1	6.9	2.9
Phosphorus + potassium	51	—	60	30	50.0	10.9	62.7
Nitrogen + phosphorus + potassium	53	—	88	49	44.3	14.2	111.9
Experiment 2†							
None—control	36	5.00	132	28	78.8	5.47	—
Triple superphosphate (1-43)	47	5.16	262	77	70.6	13.50	146.8
Ammonium phosphate (11-48)	48	5.24	298	74	75.2	15.99	192.3
Ammonium phosphate (16-20)	48	5.06	335	88	73.7	21.05	284.8
Ammonium sulphate	42	4.84	140	43	69.3	6.80	24.3

* *October 27 to December 8, 1938. Four replicates to each treatment.*
† *February 11 to March 25, 1938. Five replicates to each treatment.*

data previously published (20). Nitrogen applied singly as inorganic or organic nitrogenous fertilizer has given a slight response, and potassium no response. When phosphorus and nitrogen are applied in combination, the increase is much greater than with phosphorus alone. The nitrogen-phosphorus combination gave greatest increase, phosphorus-potassium next, and nitrogen-potassium a slight increase, indicating that phosphorus is in greatest deficiency. Once the deficiency of phosphorus is corrected, additional nitrogen gives increased yields.

It will be observed that of the materials supplying nitrogen alone, the acid nitrogenous fertilizers, ammonium nitrate, ammonium sulphate, and urea, gave slightly better response than the basic fertilizers, calcium nitrate, sodium nitrate, cyanamide, and potassium nitrate.

The complete fertilizer gave approximately the same increase as the ammonium phosphates, again indicating that ample potassium is present in infested soil.

No definite correlation is evident between the percentage of diseased crown roots and the dry weights of the plants. The impracticability of estimating accurately the amount of disease on the fine laterals of both seminal and crown roots adds to the difficulty of arriving at a disease rating that correlates closely with the amount of growth. Dry weight appears to be related to the number of healthy crown roots. Thus the 16-20 ammonium phosphate, with over three times as many healthy crown roots as the control series but with a larger percentage diseased, has produced more than twice as much plant growth.

Experiment 2. This was conducted in essentially the same manner as Experiment 1, except that the soil used had its viable *Pythium* content raised by previously growing in it a crop of wheat seedlings for five weeks, and then removing the seedlings by shaking the soil off the roots. If the next crop is sown at once before allowing the soil to dry out, an increased amount of viable inoculum is present in the fine rootlets broken off in the harvesting operations just described.

The results of Experiment 2, Table I, are similar to those in Experiment 1 except that the percentage increases from the three phosphates are greater (Figs. 1 and 2). Several factors are probably responsible for this, such as the increase in inoculum intensity, a decrease in nitrate resulting from the previous crop, and a better utilization of the phosphates because of the improved light conditions (cf. 3). There is a tendency for the phosphate to increase the number of seminal roots slightly, but not significantly.

The phosphates, especially those containing nitrogen, usually prevented, but occasionally only delayed, the appearance of leaf yellowing at the six-weeks stage when the plants were harvested. They increased the number and length of crown roots (20, p. 238), the number of tillers (Fig. 1), and the dry weights of the plants; and, in fact, gave practical control of the disease.

FIGS. 1 AND 2. *The effect of nitrogen and phosphorus on the growth of wheat in soil naturally infested with Pythium.* A, *untreated;* B, *ammonium sulphate;* C, *triple superphosphate;* D, *ammonium phosphate (11-48); and* E, *ammonium phosphate (16-20). The beneficial effect of phosphorus on the development of fine lateral roots is well brought out in Fig. 2.*

2. Phosphate Carriers

An experiment was conducted to ascertain the efficiency of various phosphate carriers as determined by the percentage increase in dry weight of fertilized plants over untreated controls in infested soil (Table II). There were four pots to each treatment. Chemically pure phosphates were used and applied at the rate of 100 lb. per acre.

The ammonium dihydrogen phosphate eliminated above-ground browning symptoms. The highly beneficial effects of this compound are due to the additional nitrogen it contains, as it has already been shown (Table I) that ammonium phosphates give the best response. Calcium as a carrier would be expected to be better than potassium, as calcium is known to inhibit the disease (Section 4 below), while potassium makes no appreciable difference (Experiment 1). Sodium appears to be a poor carrier of phosphorus for browning soils.

TABLE II

THE RELATIVE EFFECTS OF VARIOUS PHOSPHATE CARRIERS ON THE GROWTH OF WHEAT SEEDLINGS IN BROWNING SOIL

February–March, 1939

Treatment	Increase in dry weight over controls, %
Ammonium dihydrogen phosphate	125.4
Calcium dihydrogen phosphate	49.1
Potassium dihydrogen phosphate	39.0
Sodium dihydrogen phosphate	25.4

3. Phosphate Fixation

Cooke (6) has indicated that the *Pythium*-infested sugarcane soils of Hawaii are high fixers of added phosphate. Fourteen sample pairs of *Pythium*-infested wheat soils and normal soils of Saskatchewan were tested for their phosphate-fixing power. The results were irregular, neither soil series showing a greater tendency for phosphate fixation. In unpublished work, A. W. Hoffer, student assistant, has shown that whereas available phosphorus is usually lower in the infested soil (cf. 20, Part III), the total phosphorus in the two soils shows no such relation. The averages for total phosphorus were approximately the same in each series. The problem as to why diseased areas having the same total phosphorus should have more of it in an unavailable condition is one that should be given attention by the soil microbiologists and soil chemists.

4. Calcium Sulphate

Preliminary experiments had shown clearly the beneficial effects of finely ground gypsum ($CaSO_4 . 2H_2O$) in inhibiting the root rot. In Table III, data are given on the effects of gypsum and triple superphosphate singly and

TABLE III

THE EFFECT OF GYPSUM AND TRIPLE SUPERPHOSPHATE ON THE GROWTH OF WHEAT IN BROWNING SOIL

November 18 to December 30, 1938

Treatment	Dried plants	
	Weight, gm.	Increase, %
Browning soil—untreated	2.50	—
Gypsum, 0.7%	3.92	56.8
Gypsum, 1.4%	4.80	92.0
Gypsum, 2.8%	3.92	56.8
Triple superphosphate, 100 lb. per acre	4.60	84.0
Gypsum, 1.4% + triple superphosphate	7.35	194.0

TABLE IV

CHEMICAL ANALYSES OF PAIRED SAMPLES OF SOIL FROM BROWNING AND NORMAL AREAS OF WHEAT ON SUMMERFALLOWED LAND*

1937 and 1938

Sample location	Available P, p.p.m.		NO_3, p.p.m.		Exchangeable bases						Total bases, m.e.		Ca/Mg	
					Ca, m.e.		Mg, m.e.		Na, m.e.					
	Browning	Normal	Browning	Normal	Browning	Normal	Browning	Normal	Browning	Normal	Browning	Normal	Browning	Normal
1937:														
Prudhomme	52	79	38.2	30.3	16.4	28.6	13.5	14.9	—	—	—	—	1.21	1.92
Cudworth	42	67	27.8	50.5	21.2	27.6	9.5	13.1	—	—	—	—	2.23	2.10
Leofnard	56	114	18.3	9.0	15.9	16.5	7.6	4.7	—	—	—	—	2.09	3.51
Wakaw	59	77	16.2	24.7	14.1	27.0	3.3	7.9	—	—	—	—	4.27	3.42
?	54	93	29.4	13.0	24.8	37.0	9.8	9.3	—	—	—	—	2.52	3.98
St. Gregor	56	84	15.4	14.4	25.9	27.7	9.8	14.3	—	—	—	—	2.64	1.93
Composite	—	—	—	—	23.1	38.3	8.9	7.1	2.4	1.9	34.4	47.3	2.59	5.39
Plunkett	—	—	—	—	19.0	25.3	25.5	6.0	1.8	1.9	46.3	33.2	0.74	4.22
Cudworth	—	—	—	—	32.4	41.6	10.1	14.1	1.9	1.8	44.4	57.5	3.21	2.95
Wakaw	—	—	—	—	36.1	68.9	18.6	8.9	3.1	4.3	57.8	82.1	1.94	7.74
1938:														
Lanigan	66	66	5.9	1.4	26.2	17.7	7.1	7.5	1.3	1.3	34.6	26.5	3.69	2.36
Fenton	27	140	28.0	33.2	27.9	37.4	14.6	20.6	1.3	2.0	43.8	60.0	1.91	1.81
Saskatoon	60	82	5.2	3.6	15.7	17.3	11.0	11.1	1.9	1.5	28.6	29.9	1.43	1.56
Composite	46	67	41.9	18.8	22.1	29.6	8.8	12.3	1.6	1.7	32.5	43.6	2.51	2.40
Humboldt	28	35	25.7	20.2	12.2	20.6	—	—	—	—	—	—	—	—
Lanigan	50	60	2.3	1.7	7.7	17.7	—	—	—	—	—	—	—	—
Lanigan	13	22	2.6	2.4	9.6	9.6	—	—	—	—	—	—	—	—
Saskatoon	22	130	19.2	8.3	9.3	9.7	—	—	—	—	—	—	—	—
Saskatoon	59	72	11.2	14.2	8.9	21.3	—	—	—	—	—	—	—	—
Hagen	38	54	20.6	13.6	15.0	16.2	—	—	—	—	—	—	—	—
Selkirk, Man.	65	91	1.4	1.0	21.5	22.4	—	—	—	—	—	—	—	—
Mean	46.6	78.4	18.2	15.3	19.3	26.6	11.3	10.8	1.9	2.0	40.3	47.5	2.36	3.23

* *Thanks are due to G. Dion, formerly of the Soils Department, for making these determinations.*

in combination on the growth of wheat in a composite soil sample of browning soil. The gypsum was mixed into the whole mass of soil in the pots at rates of 0.7, 1.4, and 2.8% of the weight of the soil. Each treatment was in triplicate. The analysis of the untreated soil is given under browning soil in the 1938 Composite sample in Table IV.

Both the gypsum and triple superphosphate amendments gave substantial increases in the growth of wheat in browning soil. The combined amendments produced a significantly greater increase in dry weight than either component alone. Similar results were obtained by Albright and Klemme (1) from phosphates and limestone on the growth of Korean Lespedeza. These workers were of the opinion that the limestone mobilized the phosphates into the Lespedeza. Carpenter (5) has reported that calcium, as well as phosphorus, is beneficial to sugarcane in *Pythium*-infested soils in Hawaii.

The response from calcium is interesting and difficult to explain since the exchangeable calcium in browning soils, as determined by the ammonium acetate extract method, seems to be sufficient for the plants' needs (Table IV). The improvement brought about by the gypsum may possibly be due to one or more of the following: its increase in phosphate solubility, its effect on the physical condition of the soil, and its sulphur content; for, as reported later, sulphur applications are also beneficial. It is possible that the improvement in these soils derived from the commercial phosphatic amendments may be due partly to the sulphate that they contain. The results in Table II, however, show that high increases can be obtained from ammonium dihydrogen phosphate, which contains no sulphur.

It will be seen from Table IV that the average results of the analyses of 21 pairs of samples show the browning soils to contain 19.3 milliequivalents of exchangeable calcium and the normal soils 26.6 m.e. of calcium. In only one pair out of 21 is the exchangeable calcium higher in the browning soil. This is probably correlated with the usually lower available phosphorus and slightly lower pH of the infested soils (cf. 20). The results for available phosphorus and nitrate are in agreement with those reported earlier (20), and are included here for comparison with the data on exchangeable bases. No consistent differences in exchangeable magnesium, exchangeable sodium, or the calcium-magnesium ratio can be detected in the paired samples. Of the normal soils, 75% are higher in total exchangeable bases, but not appreciably.

5. Phosphate in Relation to *Pythium* Infection

It has been found extremely difficult to inoculate sterile soil uniformly with *Pythium* so that pot experiments could be conducted on a large scale, as is possible to a reasonable degree with *Fusarium* and *Helminthosporium* spp. This practically eliminates suitably replicated pot experiments to determine the specific effect of phosphate on *Pythium* infection of wheat roots. Wheat seedlings were accordingly grown in root-study boxes in sand cultures low in phosphate and high in nitrate, high in phosphate and low in nitrate, no

phosphate, and in full nutrient solutions, and the roots inoculated with agar discs of *Pythium* inoculum.

Under the conditions of the experiments the fungus attacked the roots at approximately the same rate regardless of treatment; that is, no significant differences could be recognized. It seems that the phosphate has little, if any, effect on increasing the resistance of the tissue, but that the phosphate, by stimulating root development both as regards numbers and rate of growth, helps the plants to escape infection. Thus it is found that phosphate-treated plants may have the same percentage of diseased crown roots as untreated plants in browning soil, but probably because of the larger total number and greater length of healthy crown roots, the phosphate-treated plants have been able to develop normally in spite of the diseased roots.

Greaney (9) found that deficiencies in phosphate did not affect the disease incidence of wheat plants to root rot caused by *Fusarium culmorum* (W. G. Sm.) Sacc. His results suggest that the effect of phosphatic fertilizers is more important on plant growth than on the severity of infection by *F. culmorum.* Sideris and Paxton (18) are of the opinion that resistance of pineapples to *Pythium arrhenomanes* is determined by the ability of infested plants to produce new roots and thus perpetuate the life of the plant, and susceptibility by the converse of this condition. Carpenter (5) regards susceptibility of sugarcane to *Pythium* root rot as due either to a natural weakness of the variety to attack or to a preconditioning of the roots of naturally resistant varieties by malnutrition. Certain varieties become more susceptible where the soil nitrogen is in excess, while other varieties are more readily attacked where phosphate and calcium are deficient.

6. Trace Elements

With the occasional exception of boron and zinc, trace elements have made no appreciable difference in the growth of wheat when applied to browning root-rot soil singly. In a few instances, boron, copper, manganese, and zinc in combination gave slight to moderate increases, but of a much lower order than triple superphosphate applied to the same soil. Applied individually with phosphate they all usually increase the beneficial effect of the phosphate slightly. There are probably wheat soils in the province that will be found to give definite response to one of the trace elements, but typical browning soil is not considered to be appreciably deficient in any one of the four listed above.

7. Sulphur Treatments

In small-scale pot experiments (Table V), heavy applications of finely ground sulphur gave slight to high increases in growth of wheat seedlings in browning soil, possibly owing to its indirect action on phosphatic compounds in the soil, or to its inhibitive effects on the parasites (Table VI), as much as to its deficiency as a nutrient. Sulphur amendments usually improve root development as shown in Table VI, Soil I, by an increase in length of crown

TABLE V

EFFECT OF SULPHUR ON THE YIELD OF WHEAT SEEDLINGS IN SMALL-SCALE POT EXPERIMENTS IN THREE BROWNING ROOT-ROT SOILS

Treatment	Relative yields at six-weeks stage		
	In composite 1934 soil	In composite 1935 soil	In composite 1938 soil
Browning soil—untreated	100.0	100.0	100.0
Sulphur— 400 lb. per acre	110.9	158.1	117.8
800 lb. per acre	—	167.6	—
1000 lb. per acre	—	—	208.6
1200 lb. per acre	108.0	—	—
1600 lb. per acre	107.2	—	—
2000 lb. per acre	—	—	212.1

TABLE VI

EFFECT OF SULPHUR ON THE YIELD, LESIONING, AND DEVELOPMENT OF THE CROWN ROOTS OF WHEAT SEEDLINGS IN BROWNING ROOT-ROT SOIL

Treatment	No. of plants	Crown roots				Oven-dry weight of plants, gm.	Relative yield; Control = 100
		Length, cm.	Total no.	No. healthy	Diseased, %		
Soil I: April-May, 1934							
Browning soil—untreated	20	180	60	34	43.3	2.08	100
Sulphur—400 lb. per acre	20	227	56	40	28.5	2.60	125
800 lb. per acre	20	258	60	56	6.6	2.31	111
Soil II: February-March, 1934							
Browning soil—untreated	28	—	93	9	90.3	2.88	100
Sulphur—750 lb. per acre	28	—	122	58	52.5	3.02	104.8

roots, sometimes by an increase in number (Soil II), and by a reduction in the percentage diseased. This is accompanied by improvements in the top growth and the dry weight of the plants.

The possible effect of the sulphur in the gypsum has already been referred to. Continuous grain growing draws heavily on the soil sulphur. According to Hart and Peterson (10), cereal crops remove from the soil two-thirds as much sulphur as phosphorus. Wyatt *et al.* (22) have shown that the grey podsols of Western Canada are deficient in sulphur, but most of the black and brown soils are adequately supplied with this element. Its use as a control measure for browning root rot alone is not considered to be warranted.

Triple Superphosphate and the Response of Non-cereal Crops

The following experiments (Table VII) were conducted in an attempt to obtain more information on the relative importance of *Pythium* spp. and phosphate deficiency on browning root rot. Plants requiring a high fertility level, such as carrots and lettuce (2), were grown in (i) naturally infested

browning soil, (ii) the same soil plus triple superphosphate, and (iii) normal soil. Sweet clover (12) and buckwheat (12) which are credited with the ability to feed on certain insoluble phosphatic minerals, were used in a similar experiment. Alfalfa and flax were also included. The results with carrots and lettuce are given in Experiments 1 and 2, Table VII.

TABLE VII

THE GROWTH OF CARROTS AND LETTUCE IN INFESTED, PHOSPHATE FERTILIZED, AND NORMAL SOIL

Crop	No. of replicates	Oven-dry weight			Increase over control	
		Browning soil, gm.	Browning soil + P, gm.	Normal soil, gm.	P amendment, %	Normal soil, %
Experiment 1*						
Carrots	4	2.65	—	7.57	—	185.6
Lettuce	4	3.00	—	8.48	—	182.6
Experiment 2†						
Carrots	3	1.59	3.65	2.21	129.5	38.9
Lettuce	3	2.96	6.82	4.15	130.4	40.2

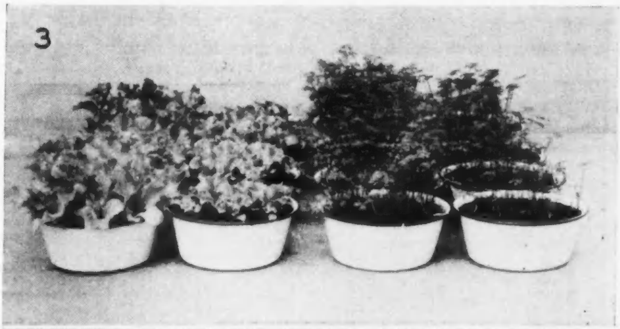
* *August 8 to October 21, 1938.*
† *October 26 to December 7, 1938.*

The carrots and the lettuce gave relatively poor growth in browning soil, good growth in normal soil, and excellent growth in browning soil to which triple superphosphate had been added (Figs. 3 and 4). The percentage increases for carrot and lettuce are virtually the same in the different tests. This indicates that the phosphate-feeding power of the two plants is similar.

For the other crops the percentage increases in air-dry weight due to the addition of triple superphosphate to browning soil were as follows: alfalfa, 41.2; buckwheat, 28.5; flax, 102.8; and sweet clover, 184.6. These experiments supply the information for which they were conducted, namely, to show whether browning soil was deficient in phosphate for non-cereal crops. For precise information on the relative response of these various crops to phosphatic fertilizers, more comprehensive tests would have to be conducted.

The roots of all these plants were free from any conspicuous lesioning such as is found on wheat seedlings grown in the same infested soil, and could be considered normal and healthy. *Pythium de Baryanum* Hesse was isolated in some instances by plating out some slightly discoloured rootlets of the carrot, lettuce, alfalfa, and sweet clover plants. In no instance was a species of *Pythium* commonly parasitic on cereals obtained. As the lesioning on the root systems of these plants was negligible, the low yield in the browning soil would appear to be due to phosphate deficiency.

With wheat, phosphate deficiency doubtless accounts for a part of the low yield in browning areas in the fallow crop, but two facts still point to the



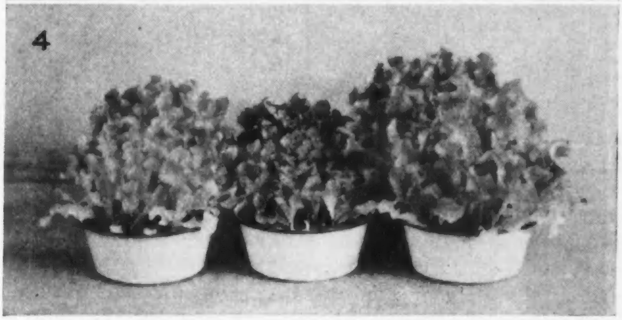


FIG. 3. *Lettuce and carrots grown in soil from normal and from browning root-rot areas of wheat fields. Normal soil is on the left and browning soil on the right of each series.*

FIG. 4. *The effect of triple superphosphate on the growth of lettuce in browning soil. Normal soil at the left; browning soil in the middle; and browning soil plus triple superphosphate at the right.*

part played by parasitic *Pythium* spp., firstly, the invariable presence of severe *Pythium* root lesioning in seedlings from such areas, and secondly, the almost universal absence of any conspicuous browning root rot in wheat on stubble, where phosphate is as low as during the fallow crop. Results reported in Part IV (20) on soil sterilization effects, indicated that root pathogens were effective in inhibiting growth and that the inhibition in browning soils could not be attributed to nutrient deficiencies alone.

Organic Amendments and Wheat Response

Improvement in the growth of wheat in browning soil from applications of cereal straw (20) suggested that information be obtained on the organic matter content of browning and normal soils. Further, as Caldwell *et al.* (4) have shown, both the nitrogen and organic matter contents of the cultivated prairie soils are being reduced by the grain and fallow system generally followed. The total nitrogen and organic carbon contents of ten paired soil samples from browning and normal areas of wheat on summer-fallowed land

have been determined, and the results are presented in Table VIII. The nitrogen was determined by the Kjeldahl procedure, and the carbon by the Walkley-Black dichromate method (21).

TABLE VIII

THE PERCENTAGES OF NITROGEN AND ORGANIC CARBON IN PAIRED SAMPLES OF BROWNING ROOT-ROT AND NORMAL SOILS*

Sample location	Total nitrogen		Organic carbon		C/N	
	Browning, %	Normal, %	Browning, %	Normal, %	Browning	Normal
1937:						
Prudhomme	.458	.460	4.11	4.10	8.97	8.91
Cudworth	.522	.543	4.63	4.65	8.87	8.51
Leofnard	.227	.206	2.45	2.15	10.80	10.44
Wakaw	.346	.369	3.14	3.22	9.08	8.73
?	.473	.269	4.26	2.47	9.00	9.18
St. Gregor	.409	.378	3.63	3.45	8.88	9.13
Engelfeldt	.471	.391	3.93	3.49	8.35	8.93
Plunkett	.267	.584	2.58	2.94	9.66	5.04
Marcelin	.389	.352	3.54	3.32	9.16	9.44
Wakaw	.447	.452	3.66	3.58	8.20	7.92
Mean	.400	.400	3.59	3.33	9.10	8.62

* *Thanks are due to G. Dion, formerly of the Soils Department, for making these determinations.*

It appears from Table VIII that there are no consistent differences in the total nitrogen, organic carbon, or C/N ratio, of browning and normal soils. The mean C/N ratio, however, is higher in the browning soils. Although there is no difference in the total nitrogen of the two soils, the nitrate nitrogen is usually slightly higher in the browning soil (Table IV, and ref. 20) at the time the disease appears.

The effects of farm manure and cereal straw. In this experiment, browning root-rot soil was filled into nine six-inch pots: three were left untreated; to another three finely divided manure was thoroughly incorporated at the rate of 15 tons per acre, and to the remaining three, chipped cereal straw at 5 tons per acre. All series were kept moist and after 10 weeks were sown to Marquis wheat. The experiment was terminated after five weeks when the control series showed severe browning symptoms. In the 30 control plants, 48 leaves were brown or yellow; in the manure series 16 were similarly discoloured, and in the straw series 26.

From the results in Table IX, it appears that the improved growth resulting from both manure and straw amendments is correlated with increased root development in which there is a higher number of roots per plant functioning in a healthy manner, and not with the percentage diseased. The washed root systems of the treated series were, however, whiter in mass than those of the controls. Improved growth is reflected in increased dry weight and a reduction in above-ground browning symptoms. These results are in confirmation of those reported earlier (20).

TABLE IX

THE EFFECTS OF MANURE AND STRAW ON THE GROWTH OF WHEAT IN NATURALLY INFESTED FIELD SOIL

January 1 to February 10, 1938

Treatment	Height, cm.	Seminal roots per plant	Crown roots			Dried plants	
			Total no.	No. healthy	Diseased, %	Weight, gm.	Increase, %
None—control	37	5.26	88	41	53.4	5.6	—
Manure	41	6.03	130	48	63.1	10.0	78.5
Straw—cereal	41	5.86	98	52	46.9	7.8	39.3

The effects of various crop residues. Freshly collected browning root-rot soil was mixed and filled into 36 one-gallon crocks sufficient for four replicates to each treatment. Farm manure at the rate of 15 tons per acre, and chipped straw, ground weed hay, and ground sweet clover hay at 3 tons per acre, were incorporated singly into the top four inches of soil of their respective series. At the same time four crocks each were sown to sweet clover and brome grass. All crocks, including 12 untreated, were kept moist. After two and a half months the sweet clover and brome plants, with as much root system as possible, were removed, ground up, and mixed into the top four inches of soil as already described. After another month all series were sown to wheat. Triple superphosphate at 200 lb. per acre and cereal straw ashes at 1000 lb. per acre were applied at the time of sowing to their respective series.

Increases in growth in the earthenware crocks follow the same trends as in pots, but are of a lower order (Table X). This is probably due to the greater room for root development, and the more compact soil with its con-

TABLE X

THE EFFECT OF ORGANIC MATTER ON THE GROWTH OF WHEAT IN BROWNING SOIL

October 25 to December 6, 1938

Treatment	Available P, p.p.m.	NO_3, p.p.m.	P/N	Crown roots			Dried plants	
				Total no.	No. healthy	Diseased, %	Weight, gm.	Increase, %
Untreated—control	42	83.3	0.50	99	61	38.3	12.9	—
Triple superphosphate	—	—	—	137	100	27.0	19.4	50.4
Farm manure	92	53.4	1.72	117	77	34.1	17.2	33.3
Cereal straw, chipped	49	41.8	1.17	76	62	18.4	14.2	10.1
Cereal straw ashes	—	—	—	128	90	29.7	15.0	16.3
Weed hay (ground)	58	100.0	0.58	111	82	26.1	15.7	21.7
Sweet clover hay (ground)	55	111.2	0.49	103	65	36.9	14.9	15.5
Sweet clover (green manure)	46	52.8	0.87	100	81	19.0	13.9	7.7
Brome grass (turned under)	52	44.8	1.16	90	76	15.0	12.9	0.0

sequent lower aeration in the crocks, both of which are known to inhibit browning root rot slightly. With the green manures of sweet clover and brome grass it is probable that insufficient time was allowed for suitable decomposition of the large amounts of fibrous material added, resulting in a deficiency of nitrogen throughout the growth period. However, with the exception of the sweet clover and brome all the other organic amendments produced a slightly beneficial carry-over effect on a second six-weeks' "wheat crop" grown in the same soil.

The slightly beneficial effect of cereal straw ashes suggests that the observed improvement from burning the stubble in browning fields is due more to the addition to the soil of the plant nutrients, especially phosphorus, which the ashes contain, than to the destruction of the parasitic fungi in the stubble. The fact that *Pythium* attacks the roots primarily, and the crowns seldom, adds further support to this view.

The available phosphorus and nitrate determinations were made at the time of sowing, but as they were determined for this single experiment only they are merely suggestive. It appears that the yield bears some relation to the phosphorus content, and little or none to the nitrate content. Owing especially to the nitrate fluctuations occurring daily in soils in pot-culture work, determinations should have been made at frequent intervals throughout the experiment.

Results to date show a constant moderate beneficial effect on wheat growth from organic matter added to browning soil.

Discussion

Of the mineral elements, phosphorus is generally recognized to be in greatest deficiency in the wheat soils of Western Canada. The rapid depletion of phosphorus in a grain system of farming is due mainly to the fact that cereal grains, which are sold off the farm, contain, according to Donaldson (7), about 75 to 85% of their total phosphorus in the seed, while phosphorus is in greater proportion in the heads than nitrogen, potassium, calcium, or sulphur. Further, as Pierre (15) has pointed out, phosphorus is found chiefly in the finest soil particles and much of it must therefore be lost by wind erosion or soil drifting. Browning root-rot soils are usually deficient in phosphorus for both cereal and non-cereal crops.

Caldwell *et al.* (4) have shown that the effects of a straight grain and fallow system on the prairie soils have been to decrease the amounts of nitrogen from 18 to 35% of the original nitrogen content as measured by the virgin sod, and to decrease the organic matter from 21 to 42% of the original organic matter content. The percentage losses increased according to soil type in ascending order as follows: black, dark brown, brown, and grey soils. The carbon was lost from the soil at a greater rate than the nitrogen. It is not surprising, therefore, that under certain conditions both nitrogenous fertilizers and organic amendments improve the growth of wheat in browning root-rot soils.

Pythium root rot is not restricted to any particular soil type, but wherever it occurs the available phosphorus is usually lower than in adjoining healthy areas. According to Russell (17) phosphate deficiency is not usually associated with any particular soil type. Also, Pierre (15) contends that it is the treatment that the soils receive after being brought under cultivation, and not the soil type, which determines the extent of phosphorus deficiency in many cases. Of all the environmental factors, phosphorus deficiency is the one most frequently associated with the increased incidence of the disease. Triple superphosphate and ammonium phosphate fertilizers, because of the production of more vigorous seedlings with a larger number of quicker growing and longer roots, give adequate control of the disease.

As recorded in the fertilizer experiments, nitrogenous compounds alone had little effect in infested soil, but once phosphate was added, further additions of nitrogen gave increases considerably higher than phosphate alone. Whereas earlier results gave no indication of any lack of nitrogen in browning soils, and indeed sometimes showed a decrease in yield when this element was applied singly, the results of the nitrogen-phosphorus combination fertilizers show that nitrogen is slightly deficient, but this deficiency is not evident as long as phosphorus is limiting. The hypothesis suggested in an earlier communication (20), that a high nitrogen-phosphorus ratio renders the plants more liable to fungal attack, would appear to be true only under conditions of low phosphorus availability. Present evidence points to the deficiency of available phosphorus as being the most important single environmental factor in the browning root-rot complex.

The work of others on the mineral nutrition of wheat may have some bearing on these results. Thus Donaldson (7) has shown that nitrate ordinarily decreases the absorption of phosphate, but that when ample phosphate is available, nitrates do not exert a depressing effect during the early periods of rapid assimilation. It is interesting to note that Carpenter (5) in Hawaii, and Le Beau (11) in the southern United States, find that nitrate applications alone increase *Pythium* root rot of sugarcane and that phosphate treatments reduce it.

The beneficial effects derived from the burning of straw are apparently due to the phosphorus which is returned to the soil and not to the destruction of the parasites in the stubble. The benefits from working in the straw are probably due to its phosphorus content as well as to stimulated biological action resulting from the fresh organic matter. The more lasting effect of turning in the straw residues, its improvement of the physical condition of the soil and its value in reducing soil erosion, make that practice more desirable than burning.

Farm manure improves seedling growth in a manner similar to that of the phosphatic fertilizers.

There is no consistent difference in the total organic matter content of browning and normal soils in the same field. Stephenson (19) has ably

emphasized the value of fresh organic matter renewal, rather than the humus level in the management of soils, and Garrett (8) has pointed out the greater effectiveness of fresh organic residues over inert humus in controlling the "take-all" disease of wheat. Several ways in which the fresh organic amendments inhibit browning root rot suggest themselves. They may result in stimulated biological activities of the soil saprophytes which would antagonize *Pythium* spp. during the active vegetative phase, or liberate nutrients from the organic matter itself, or from previously unavailable compounds in the soil. It is well known that organic materials increase the soluble phosphorus in the soil. Oberholzer (14) and Rhoades (16) in recent work incline to the view that virtually all increase in soluble phosphorus is derived from the phosphorus contained in the added material. It appears from the work of Meyer (13) that organic matter renewals are necessary for the maintenance of a good available supply of phosphorus in the soil.

The organic residues improve soil structure and reduce wind erosion, the probable deleterious effects of which on the browning root-rot situation have already been mentioned. The legumes and other non-cereal crops are primarily of value in so far as they improve soil conditions and fertility. The longevity and resistant nature of the resting spores of the parasites lessen materially the rotation value of the immunity of such plants.

These studies, together with the chemical analyses reported previously (20, Part III) on soil and plants from diseased and normal areas, suggest that browning root-rot soils have reached a point where the liberation of phosphorus and other nutrients is inadequate for crop needs. Lack of nutrient balance, as well as depletion in soil fertility, is involved. Under these conditions, the wheat seedlings are more readily attacked by several root-infecting species of *Pythium* which cannot be eradicated. It is suggested that the best means of rectifying the situation at present is by supplying supplemental nutrients in the form of artificial fertilizers, which may serve to stimulate greater biological action, and by the regular addition of organic residues or manure, which both increase nutrients and build up microbiological activities.

Acknowledgments

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A STUDY OF THE RELATION BETWEEN THE SEEDLING AND MATURE-PLANT REACTION TO *PUCCINIA GRAMINIS TRITICI* IN DURUM WHEAT CROSSES INVOLVING IUMILLO[1]

By W. H. Waddell[2]

Abstract

The seedling reaction to *Puccinia graminis Tritici*, race 21, in the greenhouse was compared with the mature-plant reaction to all races present in the field, in three intra-durum crosses involving Iumillo.

All lines that were resistant in the greenhouse were also resistant in the field. A large number of lines were susceptible at both stages. A third group contained lines susceptible in the seedling stage but resistant in the mature-plant stage. However, increasing susceptibility in the seedling stage, in general, indicated increasing susceptibility in the mature-plant stage. Most of the lines highly resistant at the mature-plant stage could have been selected on the basis of the seedling test.

Iumillo appears to possess a factor or factors for mature-plant resistance as well as a factor or factors for resistance at both stages of maturity. The reaction to stem rust in the hybrid lines did not appear to be inherited in a simple Mendelian manner. This appeared to be due, in Iumillo × Mindum crosses, to the presence of the two types of resistance in Iumillo, while in the Pentad × Iumillo crosses the inheritance was complicated still further by the additional factors for resistance in the Pentad parent.

The inheritance of seed colour was studied in a cross, Iumillo × Mindum. There appeared to be no correlation between seed colour and stem rust resistance. Seed colour in this cross was inherited in a simple 3 : 1 ratio, red-coloured seed being dominant.

The results of this study indicate that simple and inexpensive greenhouse tests, in which but one physiologic race is used, may be employed to eliminate susceptible lines in the field in durum crosses involving Iumillo.

Introduction

The extent to which a wheat cross may be tested in the greenhouse for reaction to stem rust, in order to eliminate lines susceptible in the field, is of considerable importance to the wheat breeder, especially at stations where it is difficult to obtain an adequate field epidemic.

Less labour is involved in testing the reaction of varieties to stem rust in the greenhouse than in the field. When several thousand lines are to be tested, a great deal of time and labour could be saved if simple but adequate greenhouse experiments, in which but one physiologic race is used, could be substituted for field tests.

The relation between seedling and mature-plant reaction to black stem rust in *Triticum vulgare* has received considerable attention. It has been established that the reaction to certain physiologic races in the seedling stage is not neces-

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sarily correlated with the mature-plant reaction to a number of races. Hayes and Aamodt (5) studied the inheritance of resistance to stem rust in a Marquis × Kota cross. The results indicated that certain strains were moderately resistant in the field, even though in the seedling stage they were susceptible to certain physiologic races.

Hayes, Stakman, and Aamodt (6) studied the relation between the seedling reaction and the mature-plant reaction to race 21 in the cross Marquis × Iumillo. Certain classes were obtained that were susceptible in the seedling stage to race 21 and resistant in the mature-plant stage to a number of races in the field. The Iumillo type of field resistance was found to be conditioned by at least two main factors, although modifying factors were also apparently involved.

Goulden, Neatby, and Welsh (1, 2) found that the high degree of mature-plant resistance in the strain H-44-24 was inherited entirely independently of the seedling reaction to several physiologic races. Neatby (7) reported that there was a high correlation between seedling and field reaction in the crosses Marquillo × Reward, Garnet × Marquillo, and Garnet × Double Cross. He concluded that the inheritance of the field reaction in these crosses is mainly, if not entirely, controlled by the factors that govern inheritance of the seedling reaction to race 21 in the greenhouse.

Harrington (4) studied the relation between seedling and mature-plant reaction in *T. durum*, the crosses used in this study being Kubanka No. 8 × Pentad and Mindum × Pentad. Physiologic races 1, 21, and 34 were used in the seedling studies.

The parental varieties Mindum and Pentad were tested to single races in the field by placing muslin cages over the plots, and Mindum gave the same reaction to these races in the mature-plant stage as in the seedling stage, being susceptible to both races at both stages. Pentad, on the other hand, exhibited the "mature-plant" type of resistance as reported by Goulden, Newton, and Brown (3).

Seedling reaction in the two crosses when tested with races 1, 21, and 34 appeared to depend on at least a two-factor difference. Results obtained in the Mindum × Pentad cross indicated the presence of more than one genetic factor for mature-plant reaction in the field.

In the Mindum × Pentad cross, Harrington reported a negative correlation between the seedling reaction to race 1 and the mature-plant reaction to several races in the field. No correlation was found between reaction to rust under nursery conditions and reaction to races 21 and 34 in the greenhouse in this same cross. In the Kubanka No. 8 × Pentad cross, there was no correlation between the mature-plant reaction in the field and the seedling reaction to race 34.

The durum variety Iumillo is highly resistant to stem rust, the strain used in this study being considered immune. The most extensive use of this variety in a wheat breeding program has probably been made at the Minnesota station

where a strain of Iumillo entered into the variety Marquillo and into the double cross, Thatcher.

Breeding work in which Iumillo is being used in durum crosses is under way at the Dominion Rust Research Laboratory, Winnipeg. Neatby concluded from unpublished data that there was an indication of a relation between the field reaction and the reaction in the greenhouse to physiologic race 21 in durum crosses in which Iumillo was one of the parents. The results of a further investigation of this possibility are given in this paper.

Materials and Methods

The inheritance of reaction of Iumillo to stem rust was studied in three durum wheat crosses. The first two were Iumillo × Mindum "A" and Iumillo × Mindum "B", the third being a Pentad × Iumillo cross.

Iumillo is a durum wheat of Italian origin. The strain used is immune from rust in both seedling and mature-plant stages. It is a red-seeded variety and is not of commercial importance, since the macaroni produced from it is of inferior quality.

Pentad is a durum wheat introduced from Russia by Dr. R. L. Bolley of the North Dakota Agricultural Experiment Station. It is usually resistant, having from 5 to 10% of stem rust. It is a high yielding variety, but, like Iumillo, is low in quality.

Mindum, as the name implies, was produced at the Minnesota station, University Farm. It is an amber durum selection of high macaroni quality and is moderately susceptible to stem rust.

Seedling tests of the hybrid lines and parents were made by planting about 15 seeds of each line in 4-inch pots and inoculating with race 21. Duplicate tests were made in all cases and the two results were combined. The type of pustules obtained is illustrated in Fig. 1. Classes X^- and X^+ were not included, since the slight difference between these classes and the adjacent ones would not be noticeable in a photograph.

Seedling reaction to race 21 was found to be mainly of the X type. This type of reaction was first described by Stakman and Levine (8) and is a common one in durum wheats. In the X reaction, several types of infection are to be found on the same leaf. These may range from the highly resistant 0; (zero, fleck) type to the susceptible 4 type. The reaction in the Iumillo × Mindum crosses was recorded as follows: 0;, X^{-}_{-}, X^-, X, X^+, X^{+}_{+}, and 4. In the Pentad cross, an additional class was observed. This was class 3^{+}_{-}, which is the seedling reaction of the Pentad parent.

Field reaction to a number of races in the cross Iumillo × Mindum "A" was recorded in percentage of infection, in classes of 10%, from 0 to 90. An additional class, trace (1%) was used in the other crosses.

The reaction of the parental varieties, both in seedling and mature-plant stage, is given in Table I.

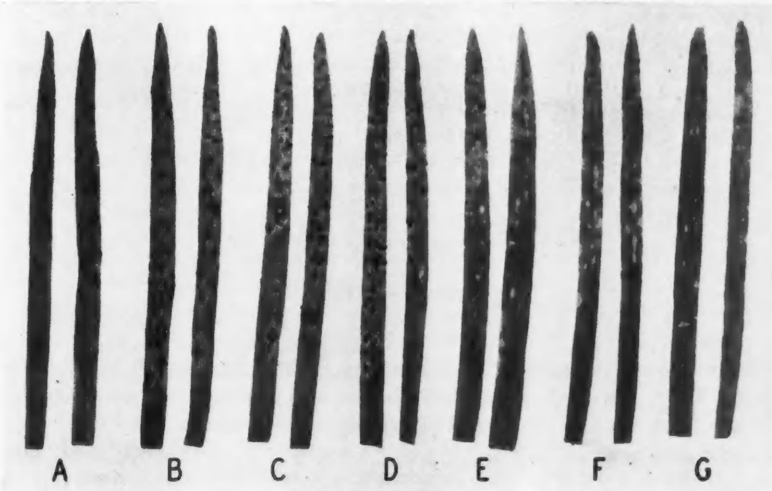

FIG. 1. *Reaction of Mindum and Iumillo parents and F_3 families in the seedling stage to physiologic race 21 in the greenhouse.* A = *Mindum*, B = *4*, C = X^{+}_{+}, D = *X*, E = X^{-}_{-}, F = *0;*, G = *Iumillo.*

TABLE I

REACTION OF PARENTAL VARIETIES IN THE SEEDLING STAGE TO RACE 21 IN THE GREENHOUSE, AND IN MATURE PLANTS TO A COLLECTION OF RACES IN THE FIELD

Parents	Seedling reaction class	Percentage of infection of mature plants
Iumillo	0;	0
Pentad	$3^{\pm}$	20*
Mindum	4	40–50

* *This reaction is unusually high for Pentad.*

The mean reaction in the seedling tests was calculated by assigning the following numerical values to the different classes in both of the Iumillo × Mindum crosses:

Numerical value	1	2	3	4	5	6
Seedling reaction	0;	X^{-}_{-}	X^{-}	X	X^{+}	X^{+}_{+} and 4

There was little, if any, difference between the field reaction of the two seedling classes X^{+}_{+} and 4. These two classes were, therefore, given the same numerical value when computing the means.

In computing the means for the percentage of infection in the field in the crosses Iumillo × Mindum "B" and Pentad × Iumillo, the following numerical values were assigned:

Numerical value	1	2	3	4	5	6	7	8	9	10	11
Percentage of infection in the field	0	1	10	20	30	40	50	60	70	80	90

The class 1% contained only plants with a slight trace of stem rust. These data show that Iumillo has an immune type of reaction to stem rust both in seedling and mature plant stage. Mindum was susceptible in the seedling stage and had 40 to 50% rust in the mature-plant stage. Pentad was slightly less susceptible in the seedling stage to race 21. This variety had a $3^{\pm}$ type of infection and showed more resistance than Mindum in the mature-plant stage.

Experimental Results

Iumillo × Mindum "A"

The first study comprised 125 plants from F_3 lines of an Iumillo × Mindum cross. These lines had been grown in the field in 1934, and notes on the mature-plant reaction to rust were obtained in classes from 0 to 90%.

These data were available at the commencement of the present study and the material was used to obtain some preliminary evidence as to the relation between seedling and mature-plant reaction to stem rust in this cross.

Approximately 15 seeds from each plant were sown in pots in the greenhouse at University Farm, St. Paul, Minnesota, in March 1936. These were inoculated with race 21 and notes taken on the basis of all plants in the pot. The basis of classification was as outlined previously.

A duplicate test of seedling reaction to race 21, from seed of the same 125 plants, was made in the greenhouse at the Dominion Rust Research Laboratory Winnipeg, in April, 1936, and the reaction determined on the same basis of classification as at University Farm. Table II shows the close relation between the two independent tests.

TABLE II

Correlation between the seedling reaction to race 21 in the cross Iumillo × Mindum "A" as determined in tests at University Farm and Winnipeg

		University Farm, St. Paul						Total
		0;	$X^{=}$	X^{-}	X	X^{+}	$X^{\ddagger}$ and 4	
Dominion Rust Research Laboratory, Winnipeg	0;	4		1				5
	$X^{=}$	8		2				10
	X^{-}	1	1	5	3	1		11
	X		1	3	7	10	4	25
	X^{+}			5	3	4	10	22
	$X^{\ddagger}$ and 4				4	8	40	52
	Total	13	2	16	17	23	54	125

A correlation coefficient of +0.82 was obtained. Only 17 plants out of a total of 125 differed by more than one class in the two independent experiments. This is a fact of considerable interest, since it shows that although the reactions dealt with were mainly of the X type, which is usually considered rather variable, a very close agreement was obtained even though the duplicate tests were made in different greenhouses at different times and under different conditions of light and temperature. It might be expected that, had the duplicate tests been made under the same conditions, an even higher correlation might have been obtained. This indicates that duplicate seedling tests can be made, with very satisfactory results, over a relatively wide variety of conditions in the Iumillo × Mindum cross.

A comparison between the F_4 seedling reaction to race 21 and the F_3 mature-plant reaction to a number of races in the field in this cross is presented in Table III. The data in this table show that a certain amount of association exists between the two reactions.

TABLE III

DISTRIBUTION OF THE MEANS OF THE F_3 LINES OF THE CROSS IUMILLO × MINDUM "A" FOR THE REACTION TO RACE 21 IN THE SEEDLING STAGE IN THE GREENHOUSE, AND THE MATURE-PLANT REACTION IN THE FIELD

Mean F_4 mature-plant reaction	Mean F_4 seedling reaction											Total
	1.0	1.1–1.5	1.6–2.0	2.1–2.5	2.6–3.0	3.1–3.5	3.6–4.0	4.1–4.5	4.6–5.0	5.1–5.5	5.6–6.0	
1	4	6	3	3	5	1	1		1		1	25
3		2			1	3	2	2	1	1		12
4						1	3	2	2	3	3	14
5						1	2	1				4
6							2	3				5
7								2		4	6	12
8							1	2	3	2	12	20
9							1		4	2	13	20
10									3	2	6	11
11										2		2
Total	4	8	3	3	6	6	12	12	14	16	41	125

Lines that were highly resistant in the seedling stage were also resistant in the mature-plant stage. A large number of lines were susceptible at both stages. However, there appeared to be a third group which was susceptible in the seedling stage but showed considerable resistance in the field. Therefore, a high correlation between the reactions at the two stages was not obtained.

IUMILLO × MINDUM "B"

In the second cross of Iumillo × Mindum "B", 303 F_3 lines were studied for reaction to stem rust. These lines were a random sample selected from the F_2 plots grown in the field in the summer of 1935 at the Dominion Rust Research Laboratory, Winnipeg, and were made available to the writer through the kindness of Dr. C. H. Goulden.

The F_2 plants were threshed and the seed divided into two lots, one for a seedling test to race 21 in the greenhouse and the remainder for a test in the mature-plant stage in the field. Duplicate plantings were made for each line in the greenhouse, the same technique being used as for Iumillo × Mindum "A". An average of about 20 seedlings of each of the 303 F_3 lines were tested in the greenhouse. The results were recorded, the scale outlined previously being used.

During the summer of 1936, the reserve seed of the 303 F_2 plants of Iumillo × Mindum "B" was planted in one-row plots in the field, in order to study the F_3 mature-plant reaction. The rows were 5 ft. long and about 25 seeds were spaced uniformly in each row.

In the fall, these one-row plots were pulled separately and notes on rust reaction were taken on each individual plant within the lines. This method would be expected to give a better estimate of the genetic composition of the F_2 plants than had the readings been made on the 303 F_2 plants themselves. This permitted a study of the association of greenhouse and field reactions based on the same generation. The basis of classification for the mature-plant reaction was the same as in the Iumillo × Mindum "B" cross, with the exception that an additional class trace (1%) was used.

The natural rust epidemic in the field during the summer of 1936 was not heavy. As, however, inoculum was placed in the field, a fairly heavy artificial epidemic was obtained. That the epidemic was not so severe as is sometimes obtained is indicated by the fact that no plants were classified as having more than 70% of stem rust. In the case of Iumillo × Mindum "A", readings as high as 90% were obtained. The Mindum parent had from 40 to 50% of rust. The strain of Mindum used in this study is one that had been selected for susceptibility as a differential host. The most prevalent races in 1936 were 21, 36, and 56.

The reactions of the parental varieties and of the F_3 lines in the field at the Dominion Rust Research Laboratory, Winnipeg, in the summer of 1936 are given in Table IV.

TABLE IV

DISTRIBUTION OF THE MEANS FOR MATURE-PLANT REACTION OF THE PARENTS AND F_3 LINES OF THE CROSS IUMILLO × MINDUM "B"

Parent or F_3 lines	Mean F_3 field reaction																	Total
	1.0	1.1–1.5	1.6–2.0	2.1–2.5	2.6–3.0	3.1–3.5	3.6–4.0	4.1–4.5	4.6–5.0	5.1–5.5	5.6–6.0	6.1–6.5	6.6–7.0	7.1–7.5	7.6–8.0	8.1–8.5	8.6–9.0	
Iumillo	67																	67
Mindum													20		22			42
F_3	7	11	9	19	27	23	30	27	28	23	21	20	17	19	11	8	3	303

The distribution of the F_3 is continuous from the reaction of the Iumillo to the reaction of the Mindum parent, even exceeding the latter in susceptibility. This would indicate that the mode of inheritance or reaction to rust

in the mature-plant stage is governed by more than one factor. It is noted that seven F_3 lines were obtained that had only immune plants like the Iumillo parent, and 11 lines that had only a trace of stem rust. From the practical standpoint, it appears that rust-resistant lines can be obtained without much difficulty.

The reaction in the seedling stage to race 21 of the 303 F_3 lines of the cross Iumillo × Mindum "B" is shown in Table V.

TABLE V

DISTRIBUTION OF THE MEANS FOR SEEDLING REACTION TO RACE 21 OF THE PARENTS AND OF THE F_3 LINES IN THE CROSS IUMILLO × MINDUM "B"

Parent or F_3 lines	Mean F_3 seedling reaction											Total
	1.0	1.1–1.5	1.6–2.0	2.1–2.5	2.6–3.0	3.1–3.5	3.6–4.0	4.1–4.5	4.6–5.0	5.1–5.5	5.6–6.0	
Iumillo	120											120
Mindum											132	132
F_3	8	4	5	10	8	18	20	31	35	48	116	303

The distribution of the F_3 lines indicates that the seedling reaction to race 21 in this cross is controlled by more than one genetic factor.

The relation between mean percentage rust infection in the field and mean pustule type of seedling infection in the greenhouse is shown in Table VI.

TABLE VI

DISTRIBUTION OF THE MEANS OF THE F_3 LINES OF THE CROSS IUMILLO × MINDUM "B" FOR THE REACTION TO RACE 21 IN THE SEEDLING STAGE IN THE GREENHOUSE, AND THE MATURE-PLANT REACTION IN THE FIELD

Mean F_3 mature-plant reaction	Mean F_3 seedling reaction											Total
	1.0	1.1–1.5	1.6–2.0	2.1–2.5	2.6–3.0	3.1–3.5	3.6–4.0	4.1–4.5	4.6–5.0	5.1–5.5	5.6–6.0	
1.0	7											7
1.1–1.5	1	3	4	3								11
1.6–2.0		1		1	2	3		1	1			9
2.1–2.5			1	3	1	2	2	4		5	1	19
2.6–3.0				1	1	6	4	3	6	1	5	27
3.1–3.5					1	2	4	2	2	5	7	23
3.6–4.0				2	2	1	2	3	7	6	7	30
4.1–4.5					1	2	4	5	4	6	5	27
4.6–5.0						1	1	6	5	6	9	28
5.1–5.5							1	4	3	6	9	23
5.6–6.0						1	1	1	2	3	13	21
6.1–6.5							1	2	4	4	9	20
6.6–7.0									1	3	13	17
7.1–7.5										1	18	19
7.6–8.0										1	10	11
8.1–8.5										1	7	8
8.6–9.0											3	3
Total	8	4	5	10	8	18	20	31	35	48	116	303

As in the case of Iumillo × Mindum "A", a high correlation between the reactions at the two stages was not obtained. However, all lines that were highly resistant to race 21 in the seedling stage were resistant in the field when inoculated with a collection of races, and a large number of lines were obtained that were susceptible at both stages. Some lines that were susceptible in the greenhouse to race 21 were resistant in the field. It may be observed, however, that as susceptibility increased in the seedling stage, there was an increase in the amount of rust found on the lines in the mature-plant stage. Seven of the 303 F_3 lines were completely free from rust, both in the seedling and in the mature-plant stage. This would indicate that, for plant breeding purposes, lines that in the seedling stage showed high resistance to race 21 would be resistant in the mature-plant stage to a number of races.

The standard deviation for field reaction of each of the 303 lines was calculated separately. The association between means and standard deviations is given in Table VII.

TABLE VII

RELATION BETWEEN MEAN AND STANDARD DEVIATION OF FIELD RUST REACTION OF F_3 LINES IN THE CROSS IUMILLO × MINDUM "B"

Mean rust classes	Standard deviation													Total
	0.0–0.25	0.26–0.50	0.51–0.75	0.76–1.00	1.01–1.25	1.26–1.50	1.51–1.75	1.76–2.00	2.01–2.25	2.26–2.50	2.51–2.75	2.76–3.00	3.01–3.25	
1.0	7													7
1.1–1.5		6	2	2	1									11
1.6–2.0				5	2	2								9
2.1–2.5		3	1	3	2	6	2	1	1					19
2.6–3.0		1	1	8	6	1	1	5	2	2				27
3.1–3.5			1		3	2	3	4	8	1	1			23
3.6–4.0					1	2	4	4	10	5	3	1		30
4.1–4.5						4	5	2	5	2	5	2	2	27
4.6–5.0					3		4	8	5	2	3	2	1	28
5.1–5.5				2	3	4	8		2	2	1	1		23
5.6–6.0			1	3	4	3	3	2	1	2	1		1	21
6.1–6.5		1	3	4	4	1	3		1	3		2		20
6.6–7.0		1	4	4	5		2			1				17
7.1–7.5	1	3	6	2	3		1	1	1	1				19
7.6–8.0	2	1	2	3	2			1						11
8.1–8.5	1	5	1	1										8
8.6–9.0	3													3
Total	14	21	22	36	38	25	36	28	36	21	14	8	4	303

It is of some interest to find that no F_3 lines with an intermediate mean reaction to rust had low standard deviations. This group exhibited greater variation between plants within a line than did the more resistant or susceptible lines. These data indicate that, in this intermediate group, there were no F_3 lines that were breeding true for reaction to rust.

Mature-plant Resistance Study

In order to test further the mature-plant resistance of Iumillo, certain lines of the cross Iumillo × Mindum "B" were studied. The mature-plant reaction of 34 F_3 lines was compared with the seedling reaction in these same lines to race 21 in the F_3 and F_4 generations.

Eighty-five plants were selected in the field from 34 F_3 lines and these plants were tested in the seedling stage in the F_4 generation to race 21. These plants were chosen mainly from the seedling susceptible-mature plant resistant group in the F_3 population, the purpose being to ascertain whether these lines that had been susceptible in the F_3 seedling stage and resistant in the F_3 mature-plant stage would show susceptibility in the F_4 seedling test. A few plants were tested also from each of the seedling resistant-mature plant resistant and seedling susceptible-mature plant susceptible groups to test the stability of these reactions.

Comparisons were made between the F_3 mature-plant reaction and the reaction in the F_3 and F_4 seedling stage. The data obtained from these comparisons are shown in Tables VIII and IX. In Table VIII, the field reaction of the single plant selections is compared with the mean seedling reaction of the line from which the plant was selected. In Table IX the mature-plant reaction of the F_3 plant selections is compared with the mean seedling reaction of the F_4 progeny.

TABLE VIII

Comparison of mature-plant reaction of selected F_3 plants with mean seedling reaction of the F_3 lines

F_3 field reaction	Mean F_3 seedling reaction											Total
	1.0	1.1–1.5	1.6–2.0	2.1–2.5	2.6–3.0	3.1–3.5	3.6–4.0	4.1–4.5	4.6–5.0	5.1–5.5	5.6–6.0	
1	6	2	1	4	1	4	1	2	4	1	2	28
2		1		1	1	3	1	2	2	6	8	25
3						1		4		4	8	17
4								1		1	5	7
5								1			2	3
6									1	1	1	3
7											1	1
8										1		1
Total	6	3	1	5	2	8	2	10	7	14	27	85

The data indicate clearly that those lines that were resistant in the field but susceptible in the seedling stage in F_3 were also susceptible in the seedling stage in the F_4 generation. This is evidence that these lines possess a type of mature-plant resistance, although in the seedling stage they are susceptible. The data also show that the F_3 seedling-resistant lines transmitted their resistance to the F_4 generation and that those lines that were susceptible in both field and greenhouse in F_3 were again susceptible in the seedling stage in the F_4 generation.

TABLE IX

COMPARISON OF MATURE-PLANT REACTION OF F_3 PLANTS WITH MEAN SEEDLING REACTION OF F_4 PROGENY

F_3 field reaction	Mean F_4 seedling reaction											Total
	1.1	1.1–1.5	1.6–2.0	2.1–2.5	2.6–3.0	3.1–3.5	3.6–4.0	4.1–4.5	4.6–5.0	5.1–5.5	5.6–6.0	
1	11	3	1	1		1	1		3	2	5	28
2	1			1	1		2		1	1	18	25
3									2	1	14	17
4											7	7
5											3	3
6										1	2	3
7											1	1
8											1	1
Total	12	3	1	2	1	1	3	0	6	5	51	85

RELATION OF STEM RUST RESISTANCE TO SEED COLOUR

Seed colour is an important factor in durum wheats; since only white-seeded varieties are suitable for the production of high quality macaroni.

Notes were taken on the seed colour of 152 F_2 lines of the Iumillo × Mindum "B" cross. The relation between seed colour and stem-rust reaction in the field in F_3 is given in Table X.

TABLE X

RELATION BETWEEN MATURE-PLANT REACTION TO STEM RUST AND SEED COLOUR IN A IUMILLO × MINDUM CROSS

Seed colour of F_2 plants	Reaction to stem rust in F_3 lines*					Total
	R	MR	SR	MS	S	
White	8	2	6	5	10	31
Red	31	16	9	23	42	121
Total	39	18	15	28	52	152

* R = *resistant;* MR = *moderately resistant;* SR = *slightly resistant;* MS = *moderately susceptible;* S = *susceptible.*

The distribution given in Table X indicates that there was no association between seed colour and stem-rust reaction in this cross. A ratio of 31 white to 121 red seeded plants in F_2 was obtained. This gives a good fit to a 3 : 1 ratio, the X^2 value being 1.72. It is apparent that seed colour in the Iumillo × Mindum cross is governed by a single factor pair.

PENTAD × IUMILLO

The third cross studied, Pentad × Iumillo, included 147 F_3 lines which constituted a random sample from the F_2 population grown at the Dominion Rust Research Laboratory, Winnipeg, in the summer of 1935.

No rust notes were taken on the F_2 plants. The F_2 plants were threshed and the seed divided, one lot being used for the seedling test and the remainder for the field test. The field test was planted in one-row plots as in the former study. Notes on the mature-plant reaction were taken on each individual plant in the lines. Mean values were computed for each F_3 line. The results are given in Table XI.

TABLE XI

DISTRIBUTION OF THE MEANS FOR MATURE-PLANT REACTION OF THE PARENTS AND OF THE F_3 LINES IN THE PENTAD × IUMILLO CROSS

Parent or F_3 lines	Mean F_3 field reaction															Total
	1.0	1.1–1.5	1.6–2.0	2.1–2.5	2.6–3.0	3.1–3.5	3.6–4.0	4.1–4.5	4.6–5.0	5.1–5.5	5.6–6.0	6.1–6.5	6.6–7.0	7.1–7.5	7.6–8.0	
Iumillo	85															85
Pentad					21		38		10							67
F_3	5	31	26	25	16	6	10	6	3	5	5	3	2	2	2	147

Both parent varieties would be classed as resistant. As previously mentioned, the readings in the present study are unusually high for Pentad. The distribution of the mean reaction of the F_3 lines greatly exceeded the limit of Pentad, the less resistant of the two parents. Nineteen F_3 lines were more susceptible than the Pentad parent. Five lines were as resistant as the Iumillo parent, approximating the same ratio as obtained in the cross of Iumillo × Mindum "B". There was a preponderance of resistant F_3 lines, since both of the parents are resistant.

The relation between the mean reactions on the F_3 lines in the mature-plant stage and on the seedlings is given in Table XII.

The distribution of the F_3 lines, showing the relation between seedling and mature-plant reaction, is similar to that obtained in the cross Iumillo × Mindum "B" (see Table VI). No F_3 lines that were resistant in the seedling stage were susceptible in the field. Only two of the 147 F_3 lines were completely free from rust in both greenhouse and field tests.

If data, such as are given in Table XII, are to be used to predict rust reaction in the mature-plant stage, an arbitrary division must be made between the seedling classes. If the four classes having the lowest mean seedling reaction are selected and grown under epidemic conditions in the field, none of the plants in the 35 lines in these classes will show any appreciable degree of rust. If, in addition, the two adjacent classes are also selected for field observation, 34 additional lines will be grown and only four of these will show any con-

TABLE XII

DISTRIBUTION OF THE MEANS OF THE F_3 LINES OF THE CROSS PENTAD × IUMILLO FOR THE REACTION TO RACE 21 IN THE SEEDLING STAGE IN THE GREENHOUSE, AND THE MATURE-PLANT REACTION IN THE FIELD

Mean F_3 mature-plant reaction	Mean F_3 seedling reaction*											Total
	1.0	1.0–1.5	1.6–2.0	2.1–2.5	2.6–3.0	3.1–3.5	3.6–4.0	4.1–4.5	4.6–5.0	5.1–5.5	5.6–6.0	
1.0	2	3										5
1.1–1.5		2	3	9	5	2	3	6		1		31
1.6–2.0		1	3	8	3	4	3	1	2	1		26
2.1–2.5		1			6	6	3	7	1		1	25
2.6–3.0		1	2		2	2	4	2	1	2		16
3.1–3.5					1	1		3			1	6
3.6–4.0						1	5	2	1		1	10
4.1–4.5						1		3		2		6
4.6–5.0								1	1	1		3
5.1–5.5								2	1	2		5
5.6–6.0							1	2	1		1	5
6.1–6.5							1	1			1	3
6.6–7.0							1		1			2
7.1–7.5								1			1	2
7.6–8.0							1		1			2
Total	2	8	8	17	18	16	25	28	12	7	6	147

* *The seedling reaction class 3± was combined with the X class and given a numerical value of 4 in computing the means.*

siderable degree of rust. If this second division is made, about 38 lines that possess "mature-plant" resistance will be discarded.

It will be noted that the number of lines possessing "mature-plant" resistance in this cross is much larger, proportionally, than the similar group in the Iumillo × Mindum "B" cross. This is, no doubt, due to the additional factor or factors for "mature-plant" resistance in the Pentad parent of the Pentad × Iumillo cross.

Discussion

The elimination of susceptible lines is one of the main considerations in a wheat breeding program in which rust resistance is one of the primary objectives. Extensive field tests under artificial epidemics are laborious and expensive and at certain breeding stations are not practical. Simple greenhouse tests may be made with lower labour costs than field tests.

The results reported in this paper indicate that, in durum crosses involving Iumillo, there is a certain amount of correlation between the seedling reaction in the greenhouse and the reaction of the mature plants in the field. In each of the crosses studied, three groups were obtained. The first group was resistant at both stages and the second was susceptible at both stages. The data indicate that in these two groups the same factors control the reaction at the two stages of development.

The third group consisted of lines that were susceptible in the seedling stage but resistant as mature plants in the field. It is apparent that as the plants in these lines approach maturity a type of reaction develops that is different from that observed in the seedling stage. This is what is known as "mature-plant" resistance.

The variety Iumillo, therefore, appears to possess at least two main types of resistance. One type is similar to that reported by Neatby (7) in a study of three crosses, all of which involved Iumillo resistance. In these crosses a high correlation was found between the reaction of the seedling plants and the mature plants, and it was concluded that the field reaction was controlled, mainly, by the factors that govern the seedling reaction to race 21 in the greenhouse.

On the other hand, Iumillo appears to possess the "mature-plant" type of resistance as reported by Goulden *et al.* (1, 2). Table VI shows that about 48 out of 303 lines were susceptible in the seedling stage but were classified as resistant in the field, thereby indicating that they possess "mature-plant" resistance.

In this regard, it is interesting to note the results in the Pentad × Iumillo cross. Goulden, Newton, and Brown (3) demonstrated that Pentad possesses a type of "mature-plant" resistance similar to that of H-44-24 and Hope. If Iumillo also possesses a type of "mature-plant" resistance it would be expected that more plants would exhibit this resistance in the Pentad × Iumillo cross than in the Iumillo × Mindum cross. An examination of Tables VI and XII shows that such is the case. In the Iumillo × Mindum cross about 48 out of 303, or 15% of the lines, possessed "mature-plant" resistance, while in the Pentad × Iumillo cross about 68 out of 147, or 46% of the lines, possessed this type of resistance.

The seedling reactions in the crosses studied are mainly of the X type, which in the Iumillo × Mindum cross appears to be quite stable from one experiment to another. Any lines that receive a reading of X^{-}_{-} or X^{-} will invariably be resistant in the mature-plant stage. Lines receiving a reading of X, X^{+}, or X^{+}_{+} may be either weakly resistant or susceptible in the mature-plant stage. Nevertheless, the data show quite conclusively that as the readings increase from X up to X^{+}_{+} there is a marked increase in susceptibility in the field.

There appears to be no indication in this study that the reaction to stem rust is inherited in a simple Mendelian manner. The distribution of the F_3 lines of the Iumillo × Mindum "B" cross for rust reaction in the field, together with the distribution obtained when the standard deviation of the means for mature-plant reaction are plotted, indicates, however, that the field reaction to stem rust in this cross is not inherited in too complex a manner to be of value from a plant breeding standpoint.

Simple inheritance in the mature-plant stage of the Pentad × Iumillo cross is not indicated by the data. Simple inheritance in the seedling stage was not indicated in any of the crosses studied.

From the standpoint of plant breeding technique, the results obtained in this study have clearly demonstrated that lines that were resistant in the seedling stage to race 21 were resistant at the mature-plant stage to all races present in the field. In a breeding program involving Iumillo × Mindum crosses, it would appear that resistance can be achieved by growing a large F_2 population and then testing the seedlings from this generation to race 21 in the greenhouse. According to the results reported in this paper, from 9 to 15% of the F_3 lines would be resistant in the seedling stage and would, therefore, be resistant as mature plants as well.

This method will eliminate some lines resistant in the mature-plant stage, as a number of lines were obtained that were susceptible in the seedling stage but resistant as mature plants in the field. However, this group did not have as high a type of field resistance as those that possessed seedling resistance also.

Unpublished data* would seem to indicate that in lines of *Vulgare* type, from crosses between Iumillo and varieties of *Triticum vulgare*, two types of resistance, similar to those described above, were present. It might be noted again that in this entire study only one physiologic race was used to determine the seedling reaction in order to forecast the reaction of the mature plants in the field.

The colour of the seed is important in durum breeding, as only amber-seeded varieties produce high quality macaroni. The results of this study indicate that in Iumillo × Mindum crosses, seed colour is dependent on a single factor pair difference, with red seed dominant. There appears to be no correlation in these crosses between rust reaction and seed colour.

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CANADIAN WILTSHIRE BACON

VII. SPECIFICATION OF COLOUR AND COLOUR STABILITY[1]

BY C. A. WINKLER[2] AND J. W. HOPKINS[3]

Abstract

A method of specifying the colour of bacon by measurement of its red, green, and blue spectral components is described.

Statistical evidence, based on a total of 792 observations, is presented which indicates that although the variation of these three components of colour from sample to sample of meat is to some extent correlated, each also exhibits a significant element of independent fluctuation. It is accordingly concluded that for investigational purposes, all three components should be included in forthcoming studies of colour and colour stability, and factors influencing them, in an extended series of samples. The possibility that for routine operations such as plant control a more limited analysis might suffice, will, however, also be made the subject of inquiry.

Introduction

Owing to the economic importance of the colour and colour stability of bacon exported from Canada, a study of these properties was included in a recent investigation (1) of some of the factors influencing Wiltshire bacon quality.

By visual inspection alone, it is possible to recognize considerable variation in the colour of different bacon samples, even when drawn from a single packing plant. In this case, the observed variation is probably due mainly to inherent differences in carcasses prior to curing. With product from different plants, the variability may be expected to be enhanced by differences in the curing practices employed. In these circumstances, it may be inferred that for an extensive study of the colour of bacon from various sources, a simple method of colour measurement, adaptable to routine procedure, is both adequate and desirable. A method by which only the red, green, and blue components of the colour are determined (5) was accordingly adopted.

Samples of meat may differ from one another in respect of either or both of two attributes of colour, namely chroma or colour quality, and total intensity or brightness. Variations in the former arise from differential reflection of one or more of the incident wave-bands by individual samples. Brightness,

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on the other hand, is determined by the ability of the meat to reflect all components present in the incident light. Actually, the analysis of samples with respect to colour quality is believed to be the more important, as hue rather than intensity of colour seems to be primarily responsible for the observed variation in the initial appearance of different sides, as well as for the grey or brown discolorations sometimes developing on exposure to air.

In this investigation the percentage reflection of each colour component, relative to that of a white reference standard, provided information respecting colour quality. Total reflection was not measured specifically, but the sum of the percentage reflections for the three components yielded some indication of brightness. Certain complications must however be recognized in the relation of this index of brightness to visual appearance. Most artificial light sources, including that used in this study, are much richer in the red component than in the green or blue. As bacon is likewise predominantly red, much more of the red than of either the green or blue components is reflected from the meat surface. This combination of low incidence of green and blue light with the low reflecting power of bacon for these colours necessitated the use of much more intense illumination in the measurement of the percentage reflection of these than was required in the case of the red component. In fact, in order to obtain comparable degrees of precision, each of the three colour bands was studied at a different intensity of white light. This does not affect the estimation of the percentage reflection of each component, which is independent of the incident intensity, but does suggest that the summation of the three percentage values on equal terms may exaggerate the effect of differences in the blue and green components on the visual appearance of samples, even in white light.

Experimental

The colour measurements to be considered in this connection were made on samples from 44 Wiltshire sides, comprising two sides from each of 22 Canadian packing plants. Three samplings were made of each side, by methods described in the introductory paper of this series (1). On each such occasion two observations, namely of initial colour at the time of sampling, and of colour stability as indicated by the changes after exposure for 20 hr. at 10° C. and 95% relative humidity, were made on each sample, also by procedures previously outlined (5). There were thus 792 observations in all.

Chroma or Colour Quality

In earlier publications (5, 6), colour quality was defined in terms of intensity ratios, namely, red : green, red : blue, and green : blue. Statistical analysis of this property is, however, more simply accomplished by a consideration of the variance and covariance of the component intensities themselves, rather than of their ratios, comparisons of the latter being complicated by the fact that the ratios secured are not independent of the general intensity

level. This procedure will accordingly be adopted in the subsequent papers of this series.

It was not known a priori whether the measurement of all three of these components was actually necessary in order to specify the colour and colour stability of bacon. This would be the case if all three components were to vary to some extent at least independently from sample to sample of meat. On the other hand, if the fluctuations in the three components, although not necessarily of the same absolute magnitude, are nevertheless closely correlated, measurement of one component alone would suffice to specify all of them. Alternatively, an intermediate situation may be envisaged in which two of the colour components are closely associated, but the third varies semi-independently. As a first step in the examination of this question, the data accumulated were used to compute the coefficients of correlation between the red, green, and blue intensities shown in Table I.

TABLE I

COEFFICIENTS OF CORRELATION BETWEEN COMPONENT INTENSITIES OF COLOUR OF INDIVIDUAL SAMPLES

Quantities correlated	First sampling	Second sampling	Third sampling	All samplings
Initial intensity				
Red × green	+.43	+.77	+.81	+.68
Red × blue	+.56	+.42	+.53	+.51
Green × blue	+.61	+.82	+.87	+.80
Stability				
Red × green	+.05	+.20	+.92	+.85
Red × blue	−.04	+.27	+.90	+.80
Green × blue	+.57	+.72	+.94	+.91
5% point	±.30	±.30	±.30	±.16

Considering first the coefficients for initial intensity at each time of sampling, given in the upper portion of Table I, all 12 of these are statistically significant, indicating that there is some real association between the component intensities of any particular sample. It is to be observed, however, that the degree of association between both red and green, and red and blue, is in no instance very high, and when the measurements for all samplings are considered collectively, is indeed quite moderate. Determination of red intensity alone consequently would not provide a highly accurate index of either the green or blue observed in the same sample, and vice versa. The association between green and blue is, however, appreciably stronger, whether the three samplings are considered individually or collectively.

In considering the observations of colour stability, or more correctly, instability, which is the property actually measured, it requires to be noted that the changes in colour on exposure after smoking (third sampling) were

much greater than those observed on either of the other two occasions, and consequently dominate the results when these are considered collectively. For this reason, in spite of the insignificant correlation between red and green and red and blue at the first two samplings, the results as a whole indicate an association between the changes in intensity which is moderately high in the case of red and green and red and blue, and quite high in the case of green and blue.

The question next arises as to whether the residual variance not accounted for by the foregoing correlations may be regarded as arising solely from experimental errors, or whether there are genuine independent fluctuations in the individual intensities from sample to sample. In the absence of any direct estimate of experimental error (single determinations only having been made on each sample) it is necessary to bring other evidence to bear on this point. This was done in two stages as follows:

The measurements of initial red, green, and blue intensity at all sampling times (396 observations) were first subjected to an analysis of variance (2) in which the main components of variation, arising from average differences between sides, between the intensity of the three colours, between the three sampling times, and the first-order interactions of these, were segregated from the second-order interactions of sides, sampling times, and individual colour intensities. As is pointed out by Fisher (3, sec. 41), such second-order interactions may be expected to be as a rule unimportant, and the variance apparently ascribable to them may accordingly be used to provide an estimate of experimental error, which will, however, be subject to inflation in proportion to the magnitude of any real interaction effects included in it.

When this was done, it was found that the residual variance of both green and blue, after correlation with red, was significantly greater than the foregoing interaction mean square, as judged by the usual variance ratio test (4). It would seem, therefore, that detectable variations in the intensity of blue and green, independent of red, may occur. The residual variance of blue after correlation with green did not exceed the interaction mean square, but owing to the possible inclusion of components other than error in the latter, this result is to be regarded as inconclusive, rather than definitely negative. In order to resolve this point, it is necessary to refer to computations, to be described in more detail in the next paper of this series, in which the homogeneity of the covariance of the green and blue intensity of individual samples within and between plants at each sampling time was examined in a manner described by Snedecor (4, sec. 12.3). By this means, significant variations in the blue intensity of samples from different plants, independent of concomitant variations in green, were demonstrable. It must be concluded, therefore, that there is in fact some element of independent variation in the intensity of each of the individual colours.

When examined in the same way, the measurements of change in colour on exposure yielded results paralleling the foregoing in that the residual variance of the change in both green and blue, after correlation with red, significantly exceeded the second-order interaction, and that the residual variance of change in blue after correlation with change in green did not. In this case, however, the subsequent analysis of covariance did not demonstrate significant differences in blue change between the product of different plants, independent of change in green.

In view of these facts, it would seem that in the investigation of an extended series of samples, measurements should be made in all three spectral regions in order to specify colour quality, particularly if it is desired to relate this to chemical or other factors. If only a limited number of samples is involved, the measurement of one component alone might suffice for comparative purposes at the present stage of development of the apparatus. For routine purposes such as plant control, it likewise seems probable that the determination of a single component might prove to be adequate, although a decision in this connection must depend on the correlation of the results of instrumental analyses with those of visual inspection. This point is currently receiving attention.

Colour Intensity or Brightness

As intimated above, the derivation of a satisfactory criterion of visual brightness from the instrumental readings made presents certain difficulties. As in the case of colour quality, it was not known beforehand whether the measurement of all three colour components was necessary, or whether one, or possibly two, of them would prove to be a satisfactory index of the total intensity. It is true that, in earlier papers, the relative brightness of different samples was assumed to be proportional to the intensity of red scatter alone, but the results then available were inadequate to demonstrate the small but significant element of independent fluctuation of each of the individual components described in the preceding section. On the other hand, in spite of this circumstance, certain considerations favour the use of the red intensity alone. One of these, touched upon already, is the probable tendency of visual inspection to assess the brightness from the intensity of the predominant red colour. Another is the fact that in addition to the independent differences noted above, there is also some degree of association between the intensities of the component colours of individual samples.

In the comparison of a limited number of samples, therefore, the red intensity may yield a satisfactory estimate of total brightness. If large numbers of samples are to be studied, as in the present investigation, the sum of the three components would seem to be preferable. A few measurements made with the apparatus without filters gave results about one-third the sum of the three percentage components obtained with filters, suggesting that the bright-

ness indicated by the latter is proportional to the intensity of reflection to be expected from a light source of the quality of a tungsten filament lamp. It is, of course, arguable that a still better index of brightness would result from a summation in which the three percentages were weighted in proportion to the absolute intensities of red, green, and blue characteristic of the incident light.

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CANADIAN WILTSHIRE BACON

VIII. COLOUR OF BACON AND ITS CORRELATION WITH CHEMICAL ANALYSES[1]

By C. A. Winkler[2], J. W. Hopkins[3], and M. W. Thistle[4]

Abstract

Photoelectric measurements on two factory-cured Wiltshire sides from each of 22 Canadian packing plants, sampled (i) upon receipt at the laboratory, (ii) after storage for 10 days at 1° C., and (iii) after smoking for 14 hr. at 40° C., indicated statistically significant differences between individual sides in respect of both total intensity and quality of colour, which would seem to have arisen mainly from differences between plants. The average range of variation between plants was: total intensity, 25%; red intensity, 23%; green, 30%; and blue, 35% of the mean. Differences in colour quality of two types, (i) due to variations in the component intensities which were correlated but not of the same absolute magnitude, and (ii) due to uncorrelated variation in the component intensities, were demonstrable.

Partial correlation studies led to the deduction of a moderate degree of association between colour quality, and pH and nitrite content, under the conditions of sampling (ii). Increased acidity was accompanied by an enhanced green and a depressed blue intensity. Increased nitrite content also tended to depress blue intensity, but apparently without significantly affecting the green. No correlation between colour and the salt, nitrate or moisture content of the meat was demonstrable.

Introduction

In this paper, some of the colour measurements made during the course of a survey of factors influencing the quality of Canadian Wiltshire bacon (1) will be discussed. As intimated in the preceding paper of this series (7), determinations were made on samples from two sides from each of 22 Canadian packing plants, or 44 sides in all. Each side was sampled, by the procedure described elsewhere (1) on three occasions, namely, (i) upon receipt at the laboratory, (ii) after storage for 10 days at 1° C., and (iii) after smoking for 14 hr. at 40° C. The resulting 132 samples were examined in a photoelectric colour comparator (6), by means of which separate measurements were made of the intensity of the light in the blue (4,000–4,500Å), green (4,900–5,800Å), and red (5,750–7,000Å) spectral regions reflected, or more correctly, scattered, at right angles to the surface of the meat. The results secured thus fall under the two heads of total intensity or brightness, and chroma or colour quality, of which the latter is considered to be the more important for reasons already put forward (7).

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In addition to the determination of colour at the actual time of sampling, measurements were also made of colour stability, as indicated by the change in colour of each sample after exposure for 20 hr. at 10° C. and 95% relative humidity. These will form the subject of a separate communication.

Colour Intensity or Brightness

It has already been shown (7) that there is some element of independent variation in the amounts of red, green, and blue scatter from sample to sample, and that measurements of all three should consequently be used in order to specify both total intensity and quality of colour. The figures for total intensity dealt with in this section are accordingly the sums of these three components in each case, although even these, for reasons indicated elsewhere (7), are probably not entirely satisfactory indices of visual brightness.

Analysis of Variance

The variance of the 132 observations of intensity or total brightness, as defined above, was analysed, by the procedure due to Fisher (5), into three portions, ascribable (i) to average differences, over all plants, between the three sampling occasions, (ii) to average differences, over the three samplings, between sides, and (iii) to the differential variation, or interaction, of sides with sampling time. The variance between sides was further allocated within and between plants, following which a corresponding partition of the interaction variance was also made. The results of this computation are shown in Table I, while Table II summarizes some of the main features of the actual observations.

TABLE I

ANALYSIS OF VARIANCE OF COLOUR INTENSITY OR BRIGHTNESS

Variance due to	Degrees of freedom	Mean square
Sides	43	76.3*
Between plants	21	97.2
Within plants	22	56.3
Sampling times	2	647.1**
Interaction sides × samplings	86	47.1
Between plants	42	55.0
Within plants (residual)	44	39.6

* *Exceeds mean square residual, 5% level of significance.*
** *Exceeds mean square residual, 1% level of significance.*

The analysis of variance indicates a definitely significant difference between sampling times in the average measurements for all 44 sides, due, however, almost entirely to the increased reflectivity of the samples taken after smoking (sampling (iii)). Moderately significant differences in the average bright-

TABLE II

SUMMARY OF MEASUREMENTS OF COLOUR INTENSITY OR BRIGHTNESS

Quantity	Sampling time		
	(i)	(ii)	(iii)
Maximum			
Individual side	104.5	102.5	110.7
Plant (av. 2 sides)	96.2	97.4	108.6
Minimum			
Individual side	74.8	74.2	72.9
Plant (av. 2 sides)	79.4	75.4	78.2
Mean	87.8	88.1	94.6

ness over all three samplings of individual sides are also demonstrable. These would seem to have arisen mainly from differences between plants, as although the number of observations available is not sufficient to establish the statistical reality of the difference in the inter- and intra-plant variance, the variance between sides drawn from the same plant does not significantly exceed the residual. This is a point of some interest, since the variability of sides from the same plant probably arises mainly from inherent differences in carcasses prior to curing, whereas between plants, differences in curing practice may be operative. On the other hand, there seems to have been no pronounced differential effect of smoking on the total colour intensity of the product from different plants, the interaction of plants $\times$ samplings (Table I), although suggestive, failing significantly to exceed the residual variance.

The means for the three samplings, shown in Table II, all fall approximately midway between the respective maximum and minimum plant averages, indicating that the values for the 22 individual plants were reasonably symmetrically distributed over the observed range of variation, without any marked bias in either direction. The average range between the maximum and minimum plant averages in the three samplings was 25% of the mean. Although doubtless susceptible of some improvement, this may perhaps be regarded as not unreasonable in view of the fact that the averages were, after all, deduced from only two sides from each plant, and further that the product of different plants was of varying age when received at the laboratory.

Correlation with Chemical Analyses

In order to ascertain whether there was any readily demonstrable association between total intensity of colour and chemical composition as determined by Cook *et al.* (2, 3), a number of simple correlation coefficients were calculated. With one exception, these proved to be uniformly insignificant. The results for sampling (iii), i.e., after smoking, yielded a coefficient of $r = -0.38$ between colour intensity and nitrite concentration in p.p.m. (5% point, $r = \pm 0.30$), but the coefficients for both the earlier samplings were insig-

nificant. Nitrate and moisture content also gave insignificant coefficients for all three samplings. Salt concentration and pH were examined only for the first two samplings. Neither was correlated with intensity. The partial correlation between intensity and pH, after allowing for associated variations in nitrite concentration, was likewise insignificant for both samplings (i) and (ii).

Chroma or Colour Quality

Analysis of Variance and Covariance of Component Intensities

Table III gives the results of an analysis of variance of the component red, green, and blue intensities of the individual samples paralleling that for total brightness. The previously noted difference in total intensity between sampling times is seen to be the result of variation in all three components. Table IV shows, however, that the increase after smoking was greatest in the blue region of the spectrum and least in the red. The analysis of variance likewise indicates that the observed differences between sides in respect of red scatter were no greater than would be expected from the residual variance in this respect of successive samples from the same side, which latter, however, is sensibly greater than the corresponding residual variance of either green or blue scatter. On the other hand, the differences between sides in both green and blue are quite significant, and the inter-plant variance of blue demonstrably exceeds the intra-plant. It is also to be noted that the increase in intensity of green and blue after smoking was not of the same magnitude in the product from all plants, the interaction of plants × samplings (Table III) being significant in both instances. A complicating factor must be noted here, however, in that the sides from the eastern and western plants were smoked on different occasions, and apparently under somewhat different conditions.

TABLE III

ANALYSIS OF VARIANCE OF COMPONENT INTENSITIES OF COLOUR

Variance due to	D.f.	Mean square		
		Red	Green	Blue
Sides	43	10.6	8.3**	13.8**
Between plants	21	11.3	10.6	24.0
Within plants	22	9.7	6.0	4.1
Sampling times	2	34.9*	68.7**	122.4**
Interaction sides × samplings	86	10.2	5.3	5.1**
Between plants	42	9.7	6.9*	8.3**
Within plants (residual)	44	10.6	3.8	1.9

* *Exceeds mean square residual, 5% level of significance.*

** *Exceeds mean square residual, 1% level of significance.*

TABLE IV

SUMMARY OF MEASUREMENTS OF COMPONENT INTENSITIES OF COLOUR

Quantity	Sampling (i)			Sampling (ii)			Sampling (iii)		
	Red	Green	Blue	Red	Green	Blue	Red	Green	Blue
Maximum									
Individual side	48.6	32.7	29.2	46.6	30.1	30.0	44.4	34.0	33.3
Plant (av. of 2 sides)	41.2	31.2	26.2	43.8	29.0	29.0	43.2	32.9	32.6
Minimum									
Individual side	30.4	22.1	20.0	30.2	22.0	19.9	32.9	21.9	20.0
Plant (av. of 2 sides)	33.0	23.4	20.6	33.2	22.2	19.9	34.0	23.3	20.9
Mean	38.0	26.6	23.2	37.8	26.1	24.1	39.4	28.5	26.4

The means of each component intensity again fall about mid-way between the respective maximum and minimum plant averages in all three samplings. The range between plants, averaged over the three samplings, is 23% in red, 30% in green, and 35% in blue.

Reference has already been made (7) to the covariance of the component intensities from sample to sample of meat. In view of this, two types of variation in colour quality are possible: (i) within a homogeneous system, due to the fact that the increments in the component intensities associated with a given increase in total brightness, although correlated, are not of the same magnitude; and (ii) of an irregular nature, due to uncorrelated variations in the component intensities.

It was shown (7) that variations in colour quality of type (ii) were demonstrable, and in this connection an analysis of covariance was employed to establish the fact that there were significant differences in the blue intensity of the product from different plants, independent of associated variations in green, on all three sampling occasions. A further analysis of the covariance of the red and blue intensities of the six samples (from two sides at each of three sampling times) of product from each plant into portions within and between samplings (3 and 2 d.f. for each of 22 plants respectively) was also made. This indicated that there were significant differences in blue intensity, independent of red, between samplings.

These analyses also provided information respecting the effect on colour quality of the correlated, as well as the uncorrelated, variations in component intensities. Thus, the regression coefficients within plants calculated in the course of the first analysis indicated an average change of 0.67 units in blue intensity associated with unit change in green, and those within samplings determined in the second analysis, an average change of 0.31 units of blue per unit of red. It must be concluded, therefore, that variations in colour quality of both the types mentioned above occur in practice, and that those of type (ii) may be encountered either in the product of different plants, or in that of the same plant sampled before and after smoking.

Correlation of Component Intensities with Chemical Analyses

As a first step in the investigation of the relation between the component intensities of colour and the chemical properties of the meat, the simple correlation coefficients listed in Table V were determined. These indicate that above-average nitrite content was associated with below-average intensities of both green and blue. Otherwise, they are quite insignificant, with the exception of those for blue intensity and pH (sampling (ii)), and blue intensity and moisture content (all data).

TABLE V

COEFFICIENTS OF SIMPLE CORRELATION BETWEEN COMPONENT COLOUR INTENSITIES AND CHEMICAL ANALYSES OF BACON

Colour measurement	Salt content	Nitrate content	Nitrite content	pH	Moisture content
Red intensity					
sampling (i)	−.03	−.18	−.00	−.06	−.06
sampling (ii)	−.06	−.13	+.06	+.01	−.03
sampling (iii)	—	+.08	−.21	—	+.18
All data	+.05	−.05	−.05	−.02	−.12
Green intensity					
sampling (i)	−.14	−.22	−.32*	−.41	−.12
sampling (ii)	−.13	−.19	−.09	+.17	+.02
sampling (iii)	—	−.06	−.38*	—	+.16
All data	−.18	−.11	−.28**	−.09	−.20
Blue intensity					
sampling (i)	+.01	−.02	−.14	−.26	−.25
sampling (ii)	−.15	−.21	−.20	+.40**	−.02
sampling (iii)	—	−.13	−.41**	—	+.21
All data	+.05	−.07	−.29**	+.05	−.31**

* *Exceeds 5% level of significance.*
** *Exceeds 1% level of significance.*

It has to be recognized, however, that simple correlation coefficients may fail to portray adequately the relation between colour quality and chemical composition. In the first place, the former is dependent in part on the general level of intensity, owing to the differential magnitude of the correlated variations in the three spectral components. Secondly, as has been pointed out by Cook and White (4), fluctuations from sample to sample in the chemical factors themselves are not all mutually independent. Both of these circumstances may operate either to obscure or to exaggerate the real relation between individual colour components and chemical qualities. For this reason, a number of third order partial correlation coefficients have been calculated, in order to examine the relation between pairs of factors independent of associated variation in certain others. These, which are confined to the results for samplings (i) and (ii), i.e., before smoking, will be found in Table VI.

TABLE VI

COEFFICIENTS OF PARTIAL CORRELATION BETWEEN COMPONENT COLOUR INTENSITIES AND CHEMICAL ANALYSES OF BACON

Quantities correlated		Independent of		Correlation coefficient	
				Sampling (i)	Sampling (ii)
Intensity	*Analysis*	*Analysis*	*Intensity*		
Red	× salt	Nitrate,	blue and green	+.03	+.03
		Nitrite,	blue and green	−.09	+.00
		Moisture,	blue and green	+.04	−.04
		pH,	blue and green	—	+.05
Red	× nitrate	Salt,	blue and green	−.18	−.01
		Nitrite,	blue and green	—	+.01
		Moisture,	blue and green	−.16	—
Red	× nitrite	Salt,	blue and green	+.17	+.10
		Nitrate,	blue and green	—	+.10
		pH,	blue and green	+.07	+.06
Red	× pH	Salt,	blue and green	—	+.12
		Nitrite,	blue and green	+.10	+.08
Red	× moisture	Salt,	blue and green	+.10	−.14
		Nitrate,	blue and green	−.00	—
Green	× salt	Nitrate,	red and blue	−.12	−.04
		Nitrite,	red and blue	−.04	−.03
		Moisture,	red and blue	−.21	−.02
		pH,	red and blue	—	−.09
Green	× nitrate	Salt,	red and blue	−.19	−.02
		Nitrite,	red and blue	—	−.02
		Moisture,	red and blue	−.26	—
Green	× nitrite	Salt,	red and blue	−.27	−.00
		Nitrate,	red and blue	—	−.01
		pH,	red and blue	−.17	+.18
Green	× pH	Salt,	red and blue	—	−.28
		Nitrite,	red and blue	−.21	−.30
Green	× moisture	Salt,	red and blue	−.10	+.15
		Nitrate,	red and blue	−.11	—
Blue	× salt	Nitrate,	red and green	+.07	−.07
		Nitrite,	red and green	+.13	−.01
		Moisture,	red and green	−.02	−.12
		pH,	red and green	—	+.05
Blue	× nitrate	Salt,	red and green	+.18	−.10
		Nitrite,	red and green	—	−.11
		Moisture,	red and green	+.10	—
Blue	× nitrite	Salt,	red and green	−.04	−.12
		Nitrate,	red and green	—	−.15
		pH,	red and green	+.07	−.35*
Blue	× pH	Salt,	red and green	—	+.45**
		Nitrite,	red and green	−.11	+.53**
Blue	× moisture	Salt,	red and green	−.21	−.17
		Nitrate	red and green	−.15	—

* *Exceeds 5% point (r = 0.31).*
** *Exceeds 1% point (r = 0.40).*

They are again, for the most part, of quite negligible magnitude. Demonstrable effects are confined to the blue region of the spectrum, in which the intensity seems to be correlated negatively with nitrite content and positively with pH, i.e., the blue component increased with increasing alkalinity. There is also some indication of a negative association between green intensity and pH, but this falls short of significance. It is noteworthy that the main colour component, red, yields no indication of association with any of the chemical quantities determined.

In view of the indications obtained, the correlation of blue and green intensities with nitrite content and pH was further investigated by the calculation of still higher order partial coefficients from the results of sampling (ii). When this was done, the fourth order coefficient for green intensity and pH, independent of red intensity, blue intensity, salt content, and nitrite content, was found to be $r = -0.33$, which just exceeds the 5% point of $r = \pm 0.31$. Similarly, the fourth order partial correlation of blue intensity and pH, independent of red intensity, green intensity, salt content, and nitrite content, gave $r = 0.56$, and the fifth order partial correlation between blue intensity and nitrite content, independent of red intensity, green intensity, salt content, nitrate content, and pH, $r = -0.43$, both of which exceed the 1% point. There would thus seem to have been, under the conditions of sampling (ii) at any rate, a moderate degree of association between these two factors and colour quality, such that increasing acidity tended to raise the green and depress the blue intensity, and increasing nitrite content also tended to depress the blue intensity, but without affecting the green.

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CANADIAN WILTSHIRE BACON

IX. COLOUR STABILITY OF BACON AND ITS CORRELATION WITH CHEMICAL ANALYSES[1]

By C. A. Winkler[2], J. W. Hopkins[3], and E. A. Rooke[4]

Abstract

Photoelectric measurements on samples of two factory-cured sides from each of 22 Canadian packing plants, sampled (i) upon receipt at the laboratory, (ii) after storage for 10 days at 1° C., and (iii) after smoking for 14 hr. at 40° C., indicated statistically significant differences between the product of individual plants in respect of the stability of total intensity of colour, and of the component intensities of red, green, and blue, on exposure of freshly-cut samples for 20 hr. at 10° C., and 95% relative humidity. Apart from a batch effect after smoking, however, there was no marked segregation of any one plant or group of plants from the remainder in respect of colour stability of product. On the average, the effect of smoking was to reduce colour stability.

Analyses of covariance demonstrated (i) a significant degree of correlation between the green and blue stability of the same sample, and (ii) a further correlation between the initial green and blue intensity and the stability of these components, samples of higher initial intensity suffering a greater reduction on exposure. Partial correlation studies suggest that increased nitrite content was accompanied by an enhanced stability of the red component of colour, but no correlation between the salt, nitrate and moisture content or pH of the meat and its colour stability was demonstrable.

In the preceding paper of this series (7), some of the colour measurements made in a survey of factors influencing the quality of Canadian Wiltshire bacon (1) were discussed. It was explained that observations were made on samples from 44 sides, two from each of 22 Canadian packing plants, each side being sampled (i) upon receipt at the laboratory; (ii) after storage for 10 days at 1° C.; and (iii) after smoking for 14 hr. at 40° C. The measurements of colour stability, now to be discussed, were secured by determining the change in intensity and quality of colour of the 132 individual samples after exposure for 20 hr. at 10° C. and 95% relative humidity. Apparatus and procedure were the same as those already described (6, 7), and the results will be dealt with under the heads previously adopted in considering the measurements of initial colour.

Change in Colour Intensity or Brightness

For reasons advanced elsewhere (6), total intensity or brightness is defined as the sum of the separate intensities of the red, green, and blue spectral components, relative to the white standard.

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Analysis of Variance

The 132 individual measurements of change in total intensity or brightness as defined above, were subjected to an analysis of variance (5), of the same form as that applied to the observations of initial colour. The results of this are given in Table I, while Table II summarizes salient features of the measurements themselves. It will be observed that the mean values in Table II are all negative in sign, indicating an average decrease in intensity of colour of the samples on exposure.

TABLE I

ANALYSIS OF VARIANCE OF CHANGE IN TOTAL INTENSITY OF COLOUR

Variance due to	Degrees of freedom	Mean square
Sides	43	39.8**
Between plants	21	71.7**
Within plants	22	9.4
Samplings	2	3064.5**
Interaction sides × samplings	86	68.9**
Between plants	42	128.3**
Within plants (residual)	44	12.2

** *Exceeds mean square residual, 1% level of significance.*

From the analysis of variance, it is to be deduced that there were significant differences in the colour stability of the product of different plants, but that the variance between sides from the same plant did not exceed the residual. On the average, stability changed significantly between samplings, but the interaction mean square indicates that the product of individual plants behaved differentially in this respect. In reality, the variance between samplings is very largely due to the decrease in average colour stability after smoking

TABLE II

SUMMARY OF MEASUREMENTS OF CHANGE IN TOTAL INTENSITY OF COLOUR

Quantity	Sampling		
	(i)	(ii)	(iii)
Maximum change			
Individual side	−12.2	−11.3	−35.7
Plant (av. 2 sides)	−10.2	− 8.0	−34.4
Minimum change			
Individual side	+ 0.1	+ 0.1	− 0.8
Plant (av. 2 sides)	− 0.1	+ 0.1	− 1.4
Mean change	− 4.7	− 1.7	−17.4

(Table II), and the differential effect is likewise largely ascribable to the fact that this decrease was confined to the sides from the eastern plants, in which it was very pronounced, and absent from those from the western plants, which were smoked in two separate batches. Unfortunately, it cannot be stated whether this discrepant behaviour was due to differences in age or other inherent properties of the meat at the time of smoking, or to variation in the conditions under which the two batches were smoked. Examination of the individual observations in samplings (i) and (ii), however, does not suggest that any one plant or group of plants stood out from the remainder in respect of colour stability of product before smoking. Rather, there was a continuous series of plant averages from the maximum to the minimum observed values.

Correlation of Change with Initial Intensity

The correlation of the observed change in total intensity of colour on exposure with the initial intensity of the freshly cut samples was also investigated, for each sampling separately. The 44 individual samples in sampling (i) gave $r = -0.28$, and those in sampling (ii) $r = -0.18$, both of which fall short of the 5% point ($r = \pm 0.30$). On the other hand, the results for sampling (iii) as a whole gave $r = +0.40$, exceeding the 1% point of $r = \pm 0.38$. This, however, was again due to the fact that the sides from the eastern plants, smoked in the first batch, exhibited both a lower average initial intensity of colour, and also an appreciably greater average decrease in intensity on exposure. So much was this the case, that when the covariance was computed about the two batch means separately, rather than about the mean of all 44 samples, a significantly negative value of $r = -0.33$ was obtained, indicating that within batches, those samples having the highest initial intensity actually tended, on the average, to show the greatest decrease on exposure.

Correlation with Chemical Analyses

Simple correlation coefficients were calculated between the change in total intensity and the salt content, nitrate content, nitrite content, moisture content, and pH of individual sides, as determined by Cook, White, and Chadderton (2, 3), but in view of the differential behaviour of the eastern and western batches after smoking, these computations were confined to the results for the first two samplings.

The resulting coefficients were all insignificant with the exception of the one for nitrite content in sampling (ii). This gave $r = +0.37$, which exceeds the 5% point of $r = 0.30$ and thus suggests that increased nitrite content may be associated with stability of colour. The corresponding value for sampling (i), $r = +0.23$, failed to attain significance, but that for sampling (ii), at $+0.39$, was practically unaffected when recomputed as a first-order partial, eliminating the effect of accompanying variations in pH.

Changes in Chroma or Colour Quality

Analysis of Variance and Covariance of Change in Component Intensities

Tables III and IV show respectively the analysis of variance and range of fluctuation of the changes on exposure, in the individual intensities of red, green, and blue. All reproduce the main features already noted in the observations of total intensity, although the differences between plants in respect of blue stability are less pronounced than those in the red and green regions. Here also the interpretation of the differential behaviour of the sides from the eastern and western plants after smoking is obscured by batch effects.

The covariance of the changes in red, green, and blue intensity in individual samples has already been discussed to some extent in an earlier paper, which contained a tabulation of the correlation coefficients bearing upon this point (6, Table I). The results for samplings (i) and (ii) agreed in indicating

TABLE III

ANALYSIS OF VARIANCE OF CHANGE IN COMPONENT INTENSITIES OF COLOUR

Variance due to	D.f.	Mean square		
		Red	Green	Blue
Sides	43	7.6**	7.0**	3.5
Between plants	21	11.2**	12.9**	5.3*
Within plants	22	4.1	1.4	1.8
Samplings	2	505.2**	390.2**	175.4**
Interaction sides × samplings	86	13.2**	9.8**	5.3*
Between plants	42	23.6**	17.5**	7.9**
Within plants (residual)	44	3.3	2.5	2.9

* *Exceeds mean square residual, 5% level of significance.*
** *Exceeds mean square residual, 1% level of significance.*

TABLE IV

SUMMARY OF MEASUREMENTS OF CHANGE IN COMPONENT INTENSITIES OF COLOUR

Quantity	Sampling (i)			Sampling (ii)			Sampling (iii)		
	Red	Green	Blue	Red	Green	Blue	Red	Green	Blue
Maximum decrease									
Individual side	−10.7	−3.8	−7.0	−6.7	−2.9	−3.5	−17.3	−11.7	−8.5
Plant (av. 2 sides)	− 7.6	−2.4	−4.0	−5.4	−1.4	−2.8	−15.9	−10.8	−8.2
Minimum decrease									
Individual side	− 0.4	+4.1	+2.6	+1.7	+2.5	+2.3	− 1.0	+ 1.7	+0.5
Plant (av. 2 sides)	− 0.8	+3.1	+1.2	+0.1	+2.0	+1.7	− 1.4	+ 0.9	+0.1
Mean change	− 3.8	−0.1	−0.8	−2.1	+0.5	−0.1	− 8.6	− 5.0	−3.8

a significant positive correlation between the changes in green and blue, but did not demonstrate any definite association of either of these quantities with red stability. Changes in all three intensities were significantly correlated in sampling (iii), but this must again be considered as a reflection of the batch effect mentioned above. It was also pointed out that there were significant variations in the stability of blue and green, independent of that of red, in the observations as a whole. This was, however, found to be no more pronounced in the sides from different plants than in those from the same plant, with the exception of green stability in sampling (i), which showed some variation from plant to plant independent of red.

A further analysis of the covariance of the red and green, and red and blue stability of the six samples (two sides at each of three sampling times) taken from the product of each plant, into portions within and between samplings, was also made. This paralleled a similar analysis of the actual intensities at the time of sampling, reported in the preceding paper (5). The results demonstrated (i) no significant element of correlation between the stability of the three colour components of individual samples of the bacon from the same plant, within samplings, but (ii) significant differences between samplings in the stability of both blue and green, independent of red stability. This effect was, however, confined to the samples from the eastern plants, and must accordingly be regarded as a further consequence of batch differences.

Correlation with Components of Initial Intensity

As in the case of total intensity, the correlation between the stability of red, green, and blue, and the initial intensity of these components of colour in the fresh samples was determined for each sampling.

The observations of initial red and change in red intensity in the two samplings prior to smoking gave $r = -0.21$ for sampling (i) and $r = +0.05$ for sampling (ii), both of which are statistically insignificant. For green, the values of r for the two samplings were -0.63 and -0.30. As the first of these exceeds the 1%, and the second attains the 5% point, both may be regarded as significant, and indicative of the fact that samples above average in initial green intensity tended to suffer a correspondingly greater reduction in this component of colour on exposure. A similar conclusion is to be drawn from the coefficients of -0.51 and -0.48 obtained from the measurements of blue intensity.

For sampling (iii), the observations in the red, green, and blue regions gave $r = -0.03$, $+0.36$, and $+0.50$ respectively over all samples, but these results were again clearly biased by batch differences, recomputations of the covariance about the batch means yielding $r = -0.31$, -0.42, and -0.39. Within batches therefore the indications are that, on the whole, greater decreases in each of the three component intensities occurred in those samples having the higher initial values.

Correlation with Chemical Analyses

In view of the anomalous circumstances attending sampling (iii), the correlation of the colour stability of individual sides with the results of chemical analyses was confined to samplings (i) and (ii). Even in these instances, it is necessary to take cognizance of possible complicating effects due to the association between the initial intensity of blue and green and the subsequent stability of these components, but it will be convenient first to examine the observed correlation between colour stability and the various chemical constituents studied, and then to determine whether any observed effects are to be regarded as due to the operation of the chemical factors directly upon stability, or indirectly through their influence on initial colour.

TABLE V

COEFFICIENTS OF SIMPLE CORRELATION BETWEEN CHANGES IN COMPONENT COLOUR INTENSITIES AND CHEMICAL ANALYSES OF BACON

Colour change	Salt content	Nitrate content	Nitrite content	pH	Moisture content
Red intensity					
Sampling (i)	+.06	+.05	−.07	−.16	−.19
Sampling (ii)	+.17	−.11	+.38*	−.09	−.06
Both samplings	+.28*	+.03	+.17	−.21*	−.32**
Green intensity					
Sampling (i)	+.19	+.10	+.36*	+.32*	+.10
Sampling (ii)	+.10	+.09	+.26	+.08	+.01
Both samplings	+.22*	+.11	+.34**	+.16	−.05
Blue intensity					
Sampling (i)	−.04	−.02	+.22	+.25	+.20
Sampling (ii)	−.02	+.06	+.25	+.04	+.32*
Both samplings	+.09	+.02	+.26*	+.09	+.05

* *Exceeds 5% level of significance.*
** *Exceeds 1% level of significance.*

Of the various simple correlation coefficients shown in Table V, those of nitrite content with green and blue stability alone exhibit any measure of persistency both within and between samplings. The apparent correlation in the data as a whole between red stability on the one hand, and salt content, pH, and moisture content on the other, is not in evidence within samplings, and hence may be due to differences in the means for the two samplings, rather than to an actual causal association. There is, however, some indication of an effect of nitrite on red stability in sampling (ii). In this, as in the other instances in which a significant effect of nitrite was demonstrable, an above average concentration of this substance was associated with increased colour stability.

TABLE VI

COEFFICIENTS OF PARTIAL CORRELATION BETWEEN STABILITY OF COMPONENT COLOUR INTENSITIES AND CHEMICAL ANALYSES OF BACON

Quantities correlated			Independent of		Correlation coefficient	
					Sampling (i)	Sampling (ii)
Change		*Analysis*	*Analysis*	*Change*		
Red	×	salt	Nitrate,	blue and green	+.02	+.15
			Nitrite,	blue and green	+.08	+.09
			Moisture,	blue and green	−.05	+.12
			pH,	blue and green	—	+.16
Red	×	nitrate	Salt,	blue and green	+.03	−.09
			Nitrite,	blue and green	—	−.05
			Moisture,	blue and green	−.03	—
Red	×	nitrite	Salt,	blue and green	−.12	+.30
			Nitrate,	blue and green	—	+.32*
			pH,	blue and green	−.00	+.39*
Red	×	pH	Salt,	blue and green	—	−.07
			Nitrite,	blue and green	−.16	−.23
Red	×	moisture	Salt,	blue and green	−.14	−.09
			Nitrate,	blue and green	−.13	—
Green	×	salt	Nitrate,	blue and red	+.23	+.18
			Nitrite,	blue and red	+.13	+.14
			Moisture,	blue and red	+.30	+.01
			pH,	blue and red	—	+.19
Green	×	nitrate	Salt,	blue and red	+.06	+.11
			Nitrite,	blue and red	—	+.09
			Moisture,	blue and red	+.15	—
Green	×	nitrite	Salt,	blue and red	+.22	+.08
			Nitrate,	blue and red	—	+.14
			pH,	blue and red	+.22	+.10
Green	×	pH	Salt,	blue and red	—	+.11
			Nitrite,		+.09	+.03
Green	×	moisture	Salt,	blue and red	+.17	−.30
			Nitrate,	blue and red	+.07	—
Blue	×	salt	Nitrate,	red and green	−.16	−.17
			Nitrite,	red and green	−.20	−.18
			Moisture,	red and green	−.10	+.06
			pH,	red and green	—	−.17
Blue	×	nitrate	Salt,	red and green	−.04	−.02
			Nitrite,	red and green	—	+.04
			Moisture,	red and green	−.01	—
Blue	×	nitrite	Salt,	red and green	+.09	+.08
			Nitrate,	red and green	—	+.04
			pH,	red and green	−.04	+.04
Blue	×	pH	Salt,	red and green	—	−.04
			Nitrite,	red and green	+.09	−.01
Blue	×	moisture	Salt,	red and green	+.07	+.45**
			Nitrate,	red and green	+.14	—

* *Exceeds 5% point (r = 0.31).*
** *Exceeds 1% point (r = 0.40).*

As was pointed out in the previously published discussion of initial colour (5), however, simple correlation coefficients may fail to provide an adequate representation of the actual underlying relations, owing to the mutual intercorrelation, in individual samples, of (i) certain of the chemical constituents (2), and (ii) of the green and blue stability, as well as (iii) of stability and initial intensity. The analysis of these relations was accordingly pursued further by the calculation of the third order partial correlation coefficients listed in Table VI. These provide a measure of the correlation between the concentration of each chemical and the stability of each component colour intensity, after making allowance for the interrelation of both quantities under consideration with the two other component intensities of colour, as well as with one additional chemical factor.

The results now suggest that the direct effect of nitrite content on colour stability was confined to the red region of the spectrum, and that the apparent correlation of this factor with green and blue stability, noted above (Table V), was due to the interrelations mentioned. The only other significant coefficient in Table VI is that between blue stability and moisture content, independent of red stability, green stability, and salt content, in sampling (ii). Salt content itself seems to have been without measurable effect on either colour or colour stability, and pH, which appeared to have a significant influence upon initial blue intensity, likewise seems to have been without effect on the subsequent stability of this component.

The foregoing inference respecting the influence of nitrite content on red change was strengthened by the calculation of the sixth order partial correlation coefficient between these quantities, independent of salt content, nitrate content, pH, initial red intensity, green change, and blue change, which was found to be 0.43. This is the more noteworthy, since the demonstrable effects of nitrite on initial colour were confined to the blue region (5). On the other hand, the apparent association between blue stability and moisture content noted above would seem to have been spurious, as the fourth order coefficient, independent of initial blue intensity, was reduced to the insignificant level of $r = -0.09$. The absence of correlation between green change and the chemical constituents studied was confirmed by the calculation of fourth order coefficients, independent of initial green intensity, which proved to be uniformly insignificant.

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THE NITROGENOUS CONSTITUENTS OF CAT'S SUBMAXILLARY SALIVA EVOKED BY PARASYMPATHETIC AND SYMPATHETIC STIMULATION[1]

By S. A. Komarov[2] and G. W. Stavraky[3]

Abstract

The nitrogenous constituents of submaxillary saliva were studied in samples of saliva secreted by cats in response (i) to chorda tympani stimulation and (ii) to administration of adrenaline. Each of these two kinds of saliva was found to contain a different and characteristic glucoprotein. These two proteins are believed to be secreted by different cells, viz., by mucous cells in response to chorda tympani stimulation, and by serous cells in response to adrenaline. A new method is described for the determination of non-protein nitrogen in submaxillary saliva. Study of the partition of non-protein nitrogen showed that urea represents quantitatively the main fraction of the non-protein nitrogen of submaxillary saliva secreted in response to either parasympathetic or sympathetic stimulation. Prolonged chorda tympani stimulation causes a decrease in the permeability of the submaxillary gland to the passage of non-protein nitrogen, the fraction least affected being the urea nitrogen. The administration of adrenaline greatly increases the permeability to all fractions of non-protein nitrogen, especially urea, and this effect persists for several hours after the adrenaline administration is discontinued.

Introduction

Data concerning the protein material and non-protein nitrogenous substances in the saliva secreted by the submaxillary glands under various conditions of stimulation are very incomplete and fragmentary. As Langley (9) states in his review of the literature on salivary secretion, the chief organic material contained in the saliva from mucous (mixed) glands is mucin. Langley considered it possible that the mucin might be present in two forms, because whereas most of the mucin is precipitated by acetic acid in a stringy lump, part of it not infrequently takes the form of fine particles. He believed that some globulin is also present in the saliva of the mixed glands. The saliva from the serous glands contains globulin, or a body allied to globulin, alkali albuminate, and a small amount of serum albumin. Almost 30 years later, in a review of the same subject, Rosemann (18) was able to add very little to the above data. To quote his own words: "It seems that the protein of the saliva belongs to the class of albumins and globulins, but exact data are lacking. Similarly we know nothing about the chemical composition of the salivary mucus." Recently Kesztyüs and Martin (8) reported that the submaxillary saliva obtained on stimulation of the chorda tympani or the sympathetic nerve in the dog contains both mucin and albumin, and that the sympathetic saliva is richer in these substances than the chorda tympani saliva.

[1] *Manuscript received February 7, 1940.*
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As to the nature of the non-protein nitrogenous constituents of the saliva, the available data relate chiefly to human saliva, and largely to the mixed saliva of the mouth cavity. The following substances have been found to be normally present in the saliva: urea, ammonia, uric acid, creatinine, amino-nitrogen. Under pathological conditions, where there is retention of non-protein nitrogenous substances in the blood, the concentration of urea, ammonia, and uric acid in the saliva is increased. A review of the rather voluminous literature on the non-protein nitrogenous constituents of saliva is given by Babkin (1, 4).

The scarcity of data concerning both the nature and the concentration of protein and non-protein nitrogenous substances in the saliva obtained by stimulation of the parasympathetic or sympathetic nerves supplying the salivary glands moved us to re-investigate this problem. Recently we have been able to obtain sufficient quantities of saliva either by parasympathetic or sympathetic stimulation of the submaxillary glands in the cat to provide some data concerning both the nature of the protein material and the partition of non-protein nitrogen. We believe that the results reported here furnish some new data concerning the composition of different types of submaxillary saliva, and moreover that they warrant important conclusions as to the part played by the parasympathetic and sympathetic nerves in the activity of the submaxillary glands.

Methods

Physiological Technique

Cats, anaesthetized with nembutal, were used in the experiments. The chorda tympani and cervical sympathetic nerves were cut on both sides and the submaxillary ducts cannulated. Rhythmic stimulation was applied to the nerves for 10-minute periods interrupted by 5-minute intervals of rest. Adrenaline was administered intravenously in some experiments as a substitute for sympathetic nerve stimulation. The physiological technique was very similar to that described in the papers of Hebb and Stavraky (7) and Langstroth, McRae, and Stavraky (10, 11). For the sake of comparison, some analyses were also made of parotid saliva, which was obtained from dogs with a permanent fistula of the parotid gland.

Chemical Analysis

For the quantitative estimation of non-protein nitrogen, the proteins of the saliva were precipitated as follows: Two volumes of acetone, containing 3% acetic acid, were thoroughly mixed with one volume of saliva. The precipitate was separated by centrifuging and washed twice with a solution consisting of two parts of acetone and one part of water, and saturated with sodium chloride. This was followed by washing with 95% alcohol, absolute acetone, a mixture of three volumes of absolute alcohol and one volume of ether, and finally with ether. The original supernatant fluid and the washings were combined and evaporated to dryness under reduced pressure

at 20°C. or below. The residue was dissolved in water so that 2 cc. of the solution was equivalent to 1 cc. of saliva. In this filtrate, urea and the nitrogenous volatile bases were determined by the urease-aeration-titration method of Van Slyke and Cullen (13, pp. 547–551) and creatine + creatinine by Folin's open flask method (13, pp. 582-585). Determination of nitrogen in the whole saliva and in the protein-free filtrates was carried out by the micro-Kjeldahl procedure of Pregl (14).

Results

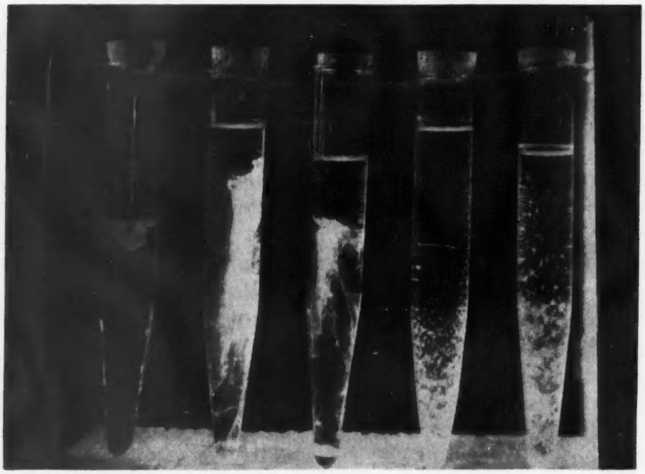
The Protein Constituents of the Submaxillary Saliva

In order to study the precipitability of the protein constituents of the saliva we have tried various known protein precipitants. Of special interest are the results obtained with trichloracetic acid and with acetone, which are presented in Table I. Acetic or hydrochloric acid in certain concentrations caused some precipitation in the submaxillary saliva obtained on stimulation of the chorda tympani, but the precipitation was never complete. The results obtained by precipitation of the saliva with two volumes of acetone containing 3% of acetic acid were of special interest. Not only was the precipitation of protein material always found to be complete, but the physical characteristics of the precipitates obtained from various kinds of submaxillary saliva, for example, (i) that secreted in response to chorda tympani stimulation (or to injection of parasympathomimetic drugs like pilocarpine), and (ii) that secreted in response to stimulation of the cervical sympathetic nerve (or to injection of adrenaline), were strikingly different. The appearance of these precipitates is illustrated in Fig. 1. The acetone precipitates obtained from sympathetic or adrenaline saliva were readily soluble in distilled water, dilute solutions of sodium carbonate, sodium and potassium hydroxide, and also in dilute solutions of hydrochloride acid, and formed non-

TABLE I

PRECIPITABILITY OF PROTEIN MATERIAL IN VARIOUS KINDS OF SALIVARY SECRETION BY TRICHLORACETIC ACID AND ACETONE IN THE PRESENCE OF ACETIC ACID

Saliva	Trichloracetic acid (5%, 7%, or 10%)	2 vol. acetone containing 3% acetic acid	Viscosity of alk., neutral, or acid soln. of acetone, ppt.
Parotid (dog)	Complete pptn.	Flocculent ppt.	Non-viscous
Submaxillary (cat), chorda tympani	No ppt.	Ppt. in form of compact viscous masses, also slight turbidity.	Viscous
Submaxillary (cat), pilocarpine	No ppt.	Ppt. in form of compact viscous masses, also slight turbidity.	Viscous
Submaxillary (cat), sympathetic	No ppt.	Flocculent ppt.	Non-viscous
Submaxillary (cat), adrenaline	No ppt.	Flocculent ppt.	Non-viscous



1 2 3 4 5

FIG. 1. *Appearance of the precipitates formed when various samples of cat's submaxillary saliva were poured into centrifuge tubes containing double the volume of 3% solution of acetic acid in acetone and the contents of each tube were thoroughly mixed by gentle rotary movements. Tubes 1 and 2, precipitates in saliva secreted in response to chorda tympani stimulation; both types—almost solid lumps or a sticky mass with numerous threads adherent to the sides—are quite typical. Tube 3, precipitate in saliva secreted in response to intravenous injection of pilocarpine; similar to that in Tube 2. Tube 4, precipitate in saliva obtained on stimulation of the cervical sympathetic nerve, and Tube 5, in saliva secreted in response to intravenous administration of adrenaline; in both, protein material was precipitated in very small and light flocculi with no tendency to adhere to the sides.*

viscous solutions. The acetone precipitates obtained from chorda tympani saliva were fairly soluble in dilute alkalis, the solutions being extremely viscous. These precipitates could also be dissolved in distilled water and in $0.5N$ hydrochloric acid, but only after many hours of vigorous stirring; the resulting solutions were very viscous.

Thus on the basis of precipitability with trichloracetic acid and with acetone it may be concluded that the parotid saliva and the parasympathetic and sympathetic submaxillary salivas have each a different protein.

A more detailed study was made of the general properties and elementary composition of the precipitates obtained by adding two volumes of acetone containing 3% of acetic acid to the submaxillary saliva secreted in response to stimulation of the chorda tympani or to injection of adrenaline. The corresponding precipitates will be referred to respectively as "Substance C" (chorda tympani saliva) and "Substance A" (adrenaline saliva).

The freshly secreted saliva was treated with acetone-acetic acid reagent immediately after collection of the sample; the precipitate was separated the following day by centrifuging, and washed twice with the same reagent diluted with one-third its volume of distilled water, twice with 95% alcohol, and finally with dry acetone and with ether. At this stage "Substance A"

was a very light, snow-white powder, readily soluble in distilled water, dilute alkalis ($0.02N$ sodium hydroxide) or dilute acids ($0.005N$ hydrochloric acid), the solutions being non-viscous. "Substance C" in appearance was a heavy, solid mass, which was very resistant to trituration in a mortar; in powder form it was completely soluble in $0.02N$ sodium hydroxide, but solution could be accomplished only after long and vigorous stirring. It was even more difficult to dissolve "Substance C" in distilled water. Its solubility in hydrochloric acid varied with the concentration; complete solution could be obtained only in concentrations slightly above $0.25N$; once solution was complete, dilution with distilled water did not cause the characteristic precipitation described by Hammarsten (6) for his preparations of mucin from the submaxillary glands. All the solutions of "Substance C" (in distilled water, sodium hydroxide, or hydrochloric acid) were very viscous. Both "Substance C" and "Substance A" could be precipitated from these solutions in dilute hydrochloric acid with four volumes of absolute alcohol, "C" in the form of sticky masses (threads or lumps), "A" in the form of light flocculi. In both cases the precipitation was complete if traces of sodium chloride were added.

In this stage of preparation both "Substance A" and "Substance C" were obtained from many samples of saliva, and they showed quite consistent percentages of nitrogen content as determined by the Kjeldahl method, viz., for "Substance A", 8.8%, and for "Substance C", 8.2%. The reducing power, which was determined by the Hagedorn-Jensen method in the products of acid hydrolysis (3 hr. at 100° C. in N sulphuric acid), was identical for both preparations, being equivalent to a 33% glucose content.

An attempt was made to purify "Substance C" further, by the method of Hammarsten (6). For this purpose about 150 mg. of the substance was finely powdered in a mortar and dissolved in a minimal amount of water with the aid of dilute hydrochloric acid. Complete solution took place when the concentration of hydrochloric acid was increased to slightly above $0.25\ N$. When the solution was diluted with water no precipitation was observed. Therefore precipitation was effected with four volumes of alcohol and traces of sodium chloride. A heavy flocculent precipitate was formed almost instantaneously; on centrifuging it became a compact, sticky mass. This was separated by centrifuging and washed several times with alcohol in increasing concentrations, and finally with absolute alcohol, alcohol-ether (3 : 1), acetone and ether, and dried to a constant weight in a vacuum desiccator. In this form the product was just as resistant to pulverization as in previous stages. In finely powdered form it appeared as a pure white and remarkably heavy substance.

"Substance A" was purified in the same manner as "Substance C". The final product was a pure white substance which, in contrast to that from "Substance C", was very light and easily pulverizable.

The solubilities of both preparations were the same as in the first stage of purification.

The general protein colour reactions were positive both with "Substance A" and "Substance C". The proteins (0.2 and 0.4% solutions in $0.03N$ sodium hydroxide or in 0.03% hydrochloric acid) were not precipitable with tungstic or phosphotungstic acid any more than with hydrochloric, sulphuric, or acetic acid at the same pH. With phosphotungstic acid the solutions of both substances showed only a very slight opalescence at pH 1. With tungstic acid the solutions of "Substance A" remained quite clear throughout the range pH 7.0 to 1.0, whereas solutions of "Substance C" showed a slight turbidity at a faintly acid reaction to litmus and a small flocculent precipitate appeared at a point at which Congo paper turns distinctly blue; precipitation was never complete, however.

The viscosity (relative to that of water at 25° C.) was determined, by Ostwald's modification of Poiseuille's method (5, p. 71), in 0.4% solutions of Substances A and C in $0.003N$ sodium hydroxide and in 0.2% solutions in dilute hydrochloric acid. Acid solutions of both substances were prepared by dilution of the alkaline solutions with equal volumes of $0.017N$ hydrochloric acid. Both solutions remained clear. The results were as follows:

Solution	Water	0.4% alkaline		Water	0.2% acid	
		A	C		A	C
Time, sec.	80	156	372	79	82	90
Viscosity	—	1.95	4.65	—	1.04	1.14

Elementary analysis of the substances (nitrogen by the micro-Dumas method, total sulphur by the micro-Carius method) was carried out in the Laboratories of the Rockefeller Institute for Medical Research in New York, through the courtesy of Dr. P. A. Levene. Table II shows the results obtained, along with the analytical data for known preparations of mucin from the submaxillary glands for the sake of comparison.

A low percentage of nitrogen (from 8.20 to 8.89%) and a high reducing power after acid hydrolysis (equivalent to 33% of glucose) were consistently found in all the preparations isolated from both chorda tympani and adrenaline submaxillary saliva. These findings indicate that the proteins of the submaxillary saliva belong to the group of glucoproteins. This conclusion is in complete agreement with general opinion. The fact that the values were identical for both the nitrogen and the reducing power in the acetone precipitates before and after purification indicates that no other proteins are present in significant quantities in the submaxillary saliva of the cat. The fact that trichloracetic acid does not cause any precipitation in submaxillary saliva suggests an even more definite conclusion, namely, that there are no other proteins, such as globulin or albumin, for example, in submaxillary saliva besides the two glucoproteins described above.

TABLE II

ELEMENTARY ANALYSIS OF VARIOUS MUCIN PREPARATIONS

	Substance A	Substance C	Scherer*	Obolenski*	Hammarsten*
C, %	—	—	52.00	52.30	48.80
H, %	—	—	6.90	7.20	6.80
N, %	8.76†	8.89†	12.80	11.90	13.30
	8.80‡	8.20‡			
S, %	1.83	2.43	—	—	0.84
Ash, %	0.85	1.25	—	—	—
Reducing power as % glucose after acid hydrolysis	32.80	32.90	—	—	—

* *The data of Scherer, Obolenski, and Hammarsten are quoted from Table XIX, p. 126, "Hexosamines and mucoproteins", by P. A. Levene (Longmans, Green & Co., London, New York, etc., 1925).*

† *Dumas method.*

‡ *Kjeldahl method.*

Thus, as shown above, "Substance A" (a protein isolated from the submaxillary saliva secreted in response to adrenaline administration) and "Substance C" (a protein isolated from the submaxillary saliva secreted in response to chorda tympani stimulation) show distinct differences in their general properties, especially with regard to the physical characteristics of the precipitates and the viscosity of their solutions. They also show significant differences in their elementary composition, particularly with regard to sulphur and nitrogen content (the latter as determined by the Kjeldahl method). Therefore these two substances must be regarded as two chemically distinct glucoproteins. This conclusion is corroborated by the recent observations of Langstroth, McRae, and Stavraky (10), who found that the extinction coefficient for chorda tympani saliva, as determined by absorption spectrum measurements, and the protein-nitrogen values differ from those for adrenaline or sympathetic saliva.

Evidence has been accumulating lately that the chorda tympani and the sympathetic nerve supply different sets of cells in the submaxillary gland, the chorda tympani supplying the mucous cells and the sympathetic the serous cells (2–4, 10, 15, 17).

Thus all the experimental evidence so far available suggests that the mucous cells, which are under the control of the chorda tympani, secrete a specific glucoprotein different from the protein material secreted by the serous cells, which are under the control of the sympathetic nerve.

It is also of interest that both these proteins differ strikingly in their elementary composition from the preparations of mucin previously isolated from the submaxillary glands (6; 12, p. 126). This suggests the possibility that during the process of secretion the glandular proteins are transformed into the different types of proteins contained in the salivary secretion.

The Non-protein Nitrogen of the Submaxillary Saliva

As follows from the previous section, the proteins of the submaxillary saliva differ from those of the blood in respect of their precipitability by various reagents. From the analytical point of view, it is important that they are not precipitable either by tungstic acid or by trichloracetic acid. Obviously none of the methods based upon the use of the above reagents are suitable for the quantitative determination of non-protein nitrogen in the submaxillary saliva. After having investigated a number of other protein precipitants we arrived at the conclusion that a very convenient and exact method of separating the protein from the non-protein fraction of the submaxillary saliva is precipitation of the saliva with two volumes of acetone, containing 3% of glacial acetic acid (described under Methods). The most convincing evidence of the completeness of fractionation by this method is the fact, repeatedly observed, that both the percentage of nitrogen in the acetone precipitate and the reducing power as determined after preliminary acid hydrolysis are constant and do not change even when the product is purified in the rather complicated manner described in the previous section. A further argument in favour of the procedure in question is that hundreds of protein nitrogen determinations by this method have been made by Langstroth, McRae, and Stavraky (10, 11), the results being checked by the spectrographic method, and it was found that they agreed well. Moreover the results thus obtained were sufficiently exact to permit of mathematical interpretation, which resulted in the development of a mathematical theory of protein secretion by the submaxillary glands (11).

Protein-free filtrates obtained by the above-described acetone method from several relatively large samples of submaxillary saliva (10.5 to 23 cc.) were analysed for their content of nitrogenous volatile bases, urea, creatine bodies, and amino-nitrogen. The results of these analyses are given in Tables III and IV; the former relates to the saliva secreted in response to chorda tympani stimulation and the latter to that obtained on administration of adrenaline.

While reserving fuller discussion of the above data for the following section, we should like to emphasize certain important observations here. Urea nitrogen is undoubtedly the main component of the non-protein fraction of the submaxillary saliva, forming from 50 to 86% of the total non-protein nitrogen. Both the absolute concentration of urea nitrogen in the saliva and the relative concentration with regard to total non-protein nitrogen are markedly higher in the adrenaline saliva and in the samples obtained by chorda tympani stimulation during the after-effect of adrenaline than the corresponding values in the chorda tympani saliva. Volatile nitrogenous bases were found to be always present in the submaxillary saliva in extremely low concentrations, namely from 0.4 to 0.7 mg. per cent nitrogen. These data are in complete agreement with the observations of Schmitz (19). Under the conditions of our experiments the variations to be found in Tables III and IV are entirely within the limits of experimental error. The amino-nitrogen

TABLE III

ANALYSIS OF SUBMAXILLARY SALIVARY SECRETION EVOKED BY RHYTHMIC STIMULATION OF THE CHORDA TYMPANI

Experiment	Sample	Stim. chorda tymp., coil cm.	Secretion		Analytical data												
			Dura-tion, min.	Rate, cc. per 10 min.	Total N, mg. %	Protein N, mg. %	NPN, mg. %	Urea N		Volatile bases N		"Creatine bodies" N		Amino-N		Uniden-tified N, % of NPN
								Mg. %	Per cent of NPN	Mg. %	Per cent of NPN	Mg. %	Per cent of NPN	Mg. %	Per cent of NPN	
No. 1, April 15 (1 gland only)	I	17	40	2.60	42.6	32.1	10.50	5.90	56.0	0.70	6.7	0.69	6.6	0.90	8.6	22.1
	II	12	30	4.40	17.5	9.1	8.40	5.00	60.0	0.40	4.8	0.60	7.0	0.90	10.7	17.5
	III	8	30	3.80	12.3	3.9	8.40	5.00	60.0	0.40	4.8	0.60	7.0	0.82	9.7	18.5
No. 2, May 8 (2 glands)	I	17	40	0.70	71.7	51.0	20.70	12.90	62.0	0.70	3.4	1.25	6.0	1.98	9.6	19.0
	II	12	30	3.05	42.6	25.8	16.80	11.40	68.0	0.60	3.6	1.19	7.0	1.70	10.0	11.4
	III	8	30	3.17	29.1	15.1	14.00	10.40	74.0	0.50	3.6	1.11	7.9	1.55	11.0	3.5
No. 3, March 10	I	12	18 hr.	1.50	—	—	9.65	6.63	68.7	0.56	5.8	0.80	8.3	0.35	3.7	13.5
Composite sample from several experiments		—	—	—	—	—	9.50	5.10	53.6	0.45	4.4	—	—	—	—	—

TABLE IV

ANALYSIS OF SUBMAXILLARY SALIVARY SECRETION EVOKED BY RHYTHMIC STIMULATION OF THE CHORDA TYMPANI OR ADMINISTRATION OF ADRENALINE

Experiment	Sample	Stimulation	Secretion		Analytical data													
			Duration, min.	Rate, cc. per 10 min.	Total N, mg. %	Protein N, mg. %	NPN, mg. %	Urea N		Volatile bases N		"Creatine bodies" N		Amino-N		Unidentified N, Per cent of NPN
								Mg %	Per cent of NPN	Mg. %	Per cent of NPN	Per cent Mg. %	Per cent of NPN	Mg. %	Per cent of NPN	
No. 5 March 24	I	Chorda tympani, coil 16 cm.	40	2.90	41.4	29.7	11.7	6.20	53.0	0.50	4.3	0.56	5.0	1.3	11.1	26.6
	II	Adrenaline	80	0.50	35.8	8.7	27.1	17.50	64.6	0.50	1.8	1.43	5.3	2.2	8.1	20.2
	III	Chorda tympani, coil 12 cm.	60	1.90	47.0	19.2	27.8	21.50	77.3	0.50	1.8	1.34	4.8	1.7	6.1	10.0
No. 6 May 27	I	Chorda tympani, coil 15 cm.	40	3.00	37.7	28.6	9.1	5.04	55.4	0.70	7.7	0.50	5.5	0.4	4.4	27.0
	II	Adrenaline	60	0.45	61.1	38.7	22.4	13.45	60.5	0.70	3.1	1.50	7.0	0.9	4.0	25.4
	III	Chorda tympani, coil 7 cm	30	2.66	58.8	36.2	22.6	16.80	74.4	0.70	3.1	1.60	7.1	0.7	3.1	12.3
Composite sample from several experiments		Adrenaline	—	—	—	—	25.8	19.00	73.6	0.52	2.0	—	—	—	—	—

values are also low, ranging from 0.4 mg. per cent to 2.2 mg. per cent. In this respect our data agree with the observations of Updegraff and Lewis (20).

In the tables the figures arbitrarily given as representing creatine + creatinine nitrogen do not represent only these substances, since the development of the colour in most cases was attained almost at once on adding alkali to the mixture of protein-free filtrate and picric acid, and furthermore the colour did not fade as quickly as in the solutions of creatinine having the same maximum colour. The concentrations of apparent creatine + creatinine were found to be from 0.5 to 0.6 mg. per cent in typical chorda tympani saliva, and were considerably greater in the samples obtained in response to injection of adrenaline, i.e., from 1.43 to 1.5 mg. per cent, and remained high in the samples obtained on chorda tympani stimulation during the after-effect of adrenaline (viz., 1.34 to 1.6 mg. per cent).

Discussion

Considerable variations were observed in the concentrations of the various nitrogenous constituents of submaxillary saliva, depending on the conditions of stimulation. A sharp progressive fall in the concentration of protein nitrogen in the successive samples of saliva obtained by rhythmic stimulation of the chorda tympani (cf. Table III) was regularly observed, which merely confirms facts established long ago. Intravenous administration of adrenaline in massive doses interposed between two periods of chorda tympani stimulation (Table IV) resulted in an increase in the concentration of total nitrogen in the chorda tympani saliva obtained after adrenaline administration. Whereas the concentration of non-protein nitrogen in this type of saliva was always greater, the percentage of protein nitrogen varied from one experiment to another. Thus, e.g., in the experiment of March 24, it was lower in the chorda tympani saliva obtained after adrenaline administration than in that obtained before, while in the experiment of May 27 it was definitely higher. These variations might be due partly to the degree of vasoconstriction produced by massive doses of adrenaline and the necessity of applying a stronger electric current to the nerve in order to obtain an adequate volume of saliva. Furthermore Langstroth, McRae, and Stavraky (10) consider that prolonged vasoconstriction produced by adrenaline administration may be partly responsible for the increased concentration of organic colloidal material in cat's submaxillary saliva obtained by subsequent stimulation of the chorda tympani.

Another conclusion which may be drawn from these experiments concerning the protein material in the submaxillary saliva is that the total output of protein material in the submaxillary saliva in response to adrenaline stimulation is not influenced by the previous activity induced in the gland by chorda tympani stimulation, but presumably depends on the store of the parent substance in the form of granule material in the serous cells. This is evident from the fact that in two experiments (Nos. 5 and 6) the rate and the duration of secretion obtained on administration of adrenaline were practically identical

(rate 0.5 and 0.45 cc. in 10 min., duration 80 and 60 min. respectively), but the concentration of protein nitrogen differed markedly in the two samples (being 8.7 and 38.7 mg. per cent). It is important to note in this connection that the preliminary secretions of the gland in response to chorda tympani stimulation were almost similar in amount in these experiments (viz., 2.9 and 3.0 cc.), that they were obtained over the same period of time (40 min.) and that they contained practically the same amount of protein (29.7 and 28.6 mg. per cent of protein nitrogen). These facts support the theory that parasympathetic and sympathetic stimulation respectively act on different cytological elements of the submaxillary gland.

Our data on non-protein nitrogen and its various fractions permit some conclusions to be drawn concerning changes in the "permeability" of the submaxillary glands to these substances under various conditions of stimulation. It was found practical for the purpose to classify the various fractions of non-protein nitrogen into three main groups:

(1) Urea nitrogen (represented in Figs. 2 and 3 by the symbol "UrN").

(2) "Creatine bodies fraction" + "volatile bases fraction" + amino-nitrogen ("RN").

(3) Unidentified nitrogen ("XN").

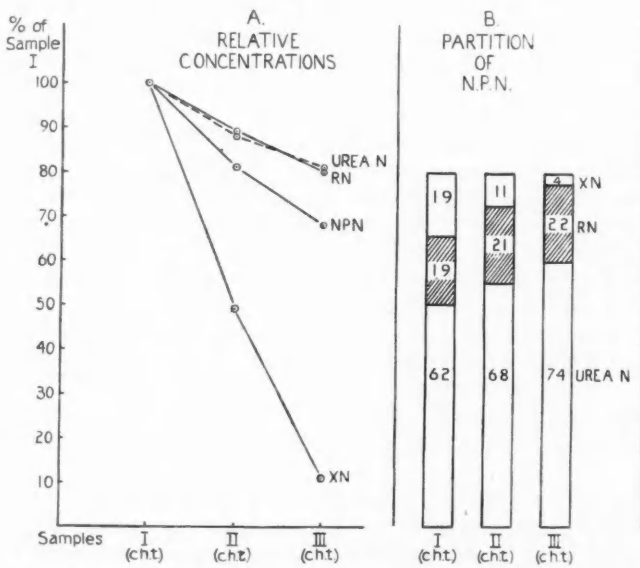


FIG. 2. *Experiment 2. Influence of chorda tympani stimulation on the non-protein nitrogen of submaxillary saliva.*

Samples I, II, and III are successive samples. (For full data see Table III.) Section A, values expressed as percentages of values for the first sample. Section B, values for three main fractions of non-protein nitrogen expressed as percentages of non-protein nitrogen. NPN, *non-protein nitrogen;* RN, *combined nitrogen of the "creatine bodies" + volatile bases + amino-nitrogen;* XN, *unidentified nitrogen.*

Although in different animals the initial values both for total non-protein nitrogen and for its fractions varied, each experiment provided convincing evidence that the concentration of certain moieties in the saliva underwent marked changes under various experimental conditions. The most probable explanation of this phenomenon is that the changes in the composition of the saliva occurred as a result of altered permeability of the cellular membranes.

Fig. 2 represents the results of two experiments in which the chorda tympani was stimulated. Section A shows changes in the concentrations of the three main fractions, expressed as percentages of the values for the first sample; Section B shows the relative concentrations of the same three fractions as percentages of the total non-protein nitrogen. In both experiments qualitatively identical results were obtained: the concentrations of total non-protein nitrogen and of each of the main fractions are lower in each successive sample; the relative concentration of "unidentified nitrogen" decreases in the successive samples to a much greater extent than that of the other fractions. Section B shows how these processes affect the partition of non-protein nitrogen in each consecutive sample. It will be seen that, as the glands secrete under the influence of the chorda tympani, an increasingly greater part of the non-protein nitrogen is formed by urea, while the fraction of the creatine bodies + volatile bases remains virtually stationary and the percentage of "unidentified nitrogen" falls considerably (especially in Experiment 2). The obvious conclusion is that continuous stimulation of the chorda tympani for several hours causes an appreciable decrease in the glandular permeability, which though it affects but slightly the passage of the small molecules such as urea (and perhaps "RN"), inhibits considerably the passage of the larger molecules such as "unidentified nitrogenous substances". This conclusion is in agreement with the results of Langstroth, McRae, and Stavraky (10), who observed a gradual decrease in the concentrations of the total anions and non-protein nitrogen in the saliva secreted under chorda tympani stimulation and ascribed this phenomenon to decrease of the glandular permeability.

Fig. 3 shows the results of Experiments 5 and 6, in which between two periods of prolonged chorda tympani stimulation (Samples I and III), the submaxillary gland was subjected for 80 and 60 min. respectively to stimulation by repeated injections of massive doses of adrenaline (Sample II). In both experiments the absolute concentration of every fraction of non-protein nitrogen was increased in the samples of adrenaline saliva, but in various degrees. In Experiments 5 and 6 respectively the concentration of non-protein nitrogen rose to 232 and 240%, that of urea nitrogen to 282 and 267%, while "RN" was 175 and 194%, and unidentified nitrogen 168 and 238%, of the initial values. After the administration of adrenaline was discontinued and chorda tympani stimulation was recommenced, the urea nitrogen continued to rise, reaching 347 and 333%, the "RN" fell slightly to 150 and 188%, while the unidentified nitrogen returned practically to the

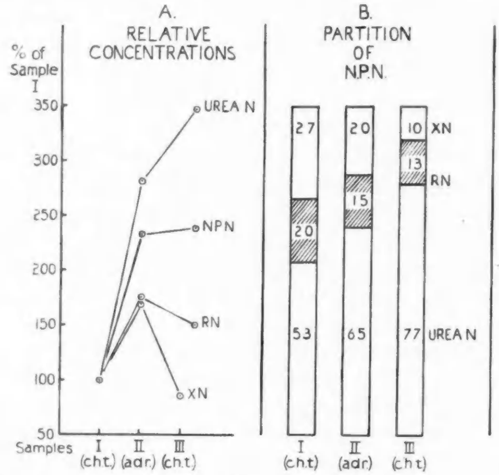


FIG. 3. *Experiment 5. Effect of adrenaline on the non-protein nitrogen of submaxillary saliva.*

Samples I (ch. t.), II (adr.), III (ch. t.) were obtained by chorda tympani stimulation, adrenaline administration, and chorda tympani stimulation immediately after discontinuing adrenaline. (See also Table IV.) Explanation of symbols as in Fig. 2.

initial values, being 85 and 114%, and the non-protein nitrogen remained stationary. Thus the administration of adrenaline increased the permeability of the gland to all fractions of non-protein nitrogen and especially to the passage of urea. The greater permeability to urea persisted or even continued to increase further for a considerable time after the administration of adrenaline was stopped and chorda tympani stimulation substituted, but the permeability to unidentified nitrogen soon returned to normal. The final result of all these phenomena was a steady and marked increase in the percentage of urea nitrogen and progressive decrease in the percentage of unidentified non-protein nitrogen, with the percentage of "RN" remaining practically stationary. The above-reported facts supplement the results of Langstroth, McRae, and Stavraky (10), who found that samples of adrenaline saliva are richer in inorganic salts, and samples of chorda tympani saliva obtained after adrenaline administration are richer in inorganic salts and glucose than samples of chorda saliva obtained before adrenaline administration. They attributed these results to the increased permeability of the glandular membrane which persists for several hours after adrenaline administration.

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